JOURNAL OF AGRICULTURAL RESEARCH

VOL. XIX WASHINGTON, D. C., JUNE 1, 1920 NO. 5

# BEHAVIOR OF THE CITRUS-CANKER ORGANISM IN THE SOIL[1]

By H. ATHERTON LEE

*Pathologist, Fruit—Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

## INTRODUCTION

It is a commonly accepted idea among fruit growers and horticulturists that the citrus-canker organism, *Pseudomonas citri* Hasse, lives and multiplies in the soil. There has been considerable field evidence to support this view. Frequently after an infected tree has been cut or burned down, young shoots have come up from the roots and have been found to be cankered. Thus Wolf [2] writes—

> That it [*P. citri*] remains alive in the soil is indicated by the appearance of diseased sprouts from the roots of diseased trees which are burned.

Stevens [3] in 1915 reported the successful cultivation of *P. citri* in sterilized soil, and this has been accepted by a number of horticulturists as sufficient evidence to conclude that the canker organism is a soil inhabitant.

The presence or absence of the canker organism in the soil is an important question, and the use of many of the eradication and quarantine methods depends upon a knowledge of the behavior of the canker organism in the soil. The question resolves itself into three points: (1) whether

[1] The investigations reported in this paper were carried on at the Lamao Agricultural Experiment Station of the Philippine Bureau of Agriculture. The writer is greatly indebted to Col. Adrian Hernandez, Director, and Mr. S Apostol, Chief, Plant Industry Division of the Philippine Bureau of Agriculture, for the facilities afforded at Lamao and for much other assistance. Thanks are also due Mr. Francisco Galang, Superintendent of the Station at Lamao, for helpfulness at all times.

Deep appreciation is here expressed to Mr. E. D. Merrill, botanist, of the Philippine Bureau of Science, for the use of the very extensive laboratory facilities in Manila and for many other kindnesses. The photographs reproduced here were taken by the photographer of the Philippine Bureau of Science from the material of the writer.

[2] WOLF, Frederick A. CITRUS CANKER. *In* Jour. Agr. Research, v. 6, no. 2, p. 69–100, 8 fig., pl. 9–11. 1916. Literature cited, p. 98–99.

[3] STEVENS, H. E. CITRUS CANKER-III. Fla. Agr. Exp. Sta. Bul. 128, 20 p., 6 fig. 1915.

*P. citri* is able to live actively—that is, increase and multiply within the soil; (2) whether it exists simply passively and does not increase and multiply; or (3) whether it is killed within the soil.

The problem has been attacked previously by the writer and other investigators by attempting to plate out soil samples and thus show the presence of the canker organism in the soil. These attempts have usually given negative results. However, such negative results have been inconclusive because of the large number of the soil organisms which would appear in the plates, making it difficult to identify *P. citri* even if it were present. Investigations were therefore undertaken at Lamao, P. I., with the purpose of attacking this question with different experimental methods.

EXPERIMENT I

Fifty-five culture tubes of orchard soil from Lamao were prepared. These were autoclaved twice, one hour each time, at 45 pounds pressure, 24 hours intervening between periods of steaming. Fifty-five tubes of the same soil were prepared but were not sterilized. Each tube of sterilized soil was inoculated with 2 cc. of a heavy infusion of *P. citri* in sterile water, precautions being used as far as possible to avoid contamination. The tubes of unsterilized soil were inoculated each with 2 cc. of the same infusion, all processes being identical except that one series of tubes was sterilized while the other was not.

Five of the sterilized tubes and 5 of the unsterilized tubes were taken; portions were removed from each with a spatula (a separate spatula being used for each tube); and infusions were then made from each of these 10 portions. These infusions were made in dry sterilized test tubes, but tap water was used for the liquid medium. Inoculations from each infusion were made upon the upper and lower surfaces of five young, actively growing pummelo[1] leaves, *Citrus maxima* (syn. *C. grandis*, *C. decumana*). Forty punctures were made in each leaf. The leaves were then bound in waxed paper with wet cotton to maintain a moist atmosphere. The whole was covered with opaque paper to prevent burning by the sun.

This procedure was repeated each day for a period of 15 days, a new series of five tubes of sterilized soil and a new series of tubes of unsterilized soil being used each day. The inoculation data and results are given in Tables I and II. The percentages expressed are based upon the number of positive takes resulting from the total of 200 punctures on 5 leaves. Where stomatal infections occur they are counted as wound infections.

[1] Following the usage of W. T. Swingle in Bailey's Standard Cyclopedia of Horticulture, the term pummelo is used in its usual East Indian sense to include varieties of *Citrus grandis* distinct from the grapefruit group of the West Indies and the United States.

TABLE I.—*Inoculations on young pummelo leaves from infusions of untreated soil in tubes made on consecutive days after inoculation with P. citri*

| Leaves No. | Infusion tube No. | Number of days after inoculation of tubes. | Date of inoculation from tubes to leaves. | Infections from 200 punctures. | Date of observation. |
|---|---|---|---|---|---|
| 1 to 5 | 1 | 2 | Oct. 23, 1918 | 93 per cent positive | Nov. 2, 1918. |
| 6 to 10 | 2 | 2 | do | 36 per cent positive | Do. |
| 11 to 15 | 3 | 2 | do | do | Do. |
| 16 to 20 | 4 | 2 | do | 22 per cent positive | Do. |
| 21 to 25 | 5 | 2 | do | 61½ per cent positive | Do. |
| 26 to 30 | 6 | 3 | Oct. 24, 1918 | 51¼ per cent positive | Nov. 4, 1918. |
| 31 to 35 | 7 | 3 | do | 6 per cent positive | Do. |
| 36 to 40 | 8 | 3 | do | 12 per cent positive | Do. |
| 41 to 45 | 9 | 3 | do | 4½ per cent positive | Do. |
| 46 to 50 | 10 | 3 | do | All negative | Do. |
| 51 to 55 | 11 | 4 | Oct. 25, 1918 | ½ of 1 per cent positive. | Do. |
| 56 to 60 | 12 | 4 | do | All negative | Do. |
| 61 to 65 | 13 | 4 | do | 3 per cent positive | Do. |
| 66 to 70 | 14 | 4 | do | ½ of 1 per cent positive. | Do. |
| 71 to 75 | 15 | 4 | do | 3 per cent positive | Do. |
| 76 to 80 | 16 | 5 | Oct. 26, 1918 | All negative | Nov. 5, 1918. |
| 81 to 85 | 17 | 5 | do | ½ of 1 per cent positive. | Do. |
| 86 to 90 | 18 | 5 | do | All negative | Do. |
| 91 to 95 | 19 | 5 | do | ½ of 1 per cent positive. | Do. |
| 96 to 100 | 20 | 5 | do | 1 per cent positive | Do. |
| 101 to 105 | 21 | 7 | Oct. 28, 1918 | All negative | Nov. 9, 1918. |
| 106 to 110 | 22 | 7 | do | do | Do. |
| 111 to 115 | 23 | 7 | do | do | Do. |
| 116 to 120 | 24 | 7 | do | do | Do. |
| 121 to 125 | 25 | 7 | do | do | Do. |
| 126 to 130 | 26 | 9 | Oct. 30, 1918 | do | Nov. 20, 1918. |
| 131 to 135 | 27 | 9 | do | do | Do. |
| 136 to 140 | 28 | 9 | do | do | Do. |
| 141 to 145 | 29 | 9 | do | 9½ per cent positive | Do. |
| 146 to 150 | 30 | 9 | do | All negative | Do. |
| 151 to 155 | 31 | 11 | Nov. 1, 1918 | do | Do. |
| 156 to 160 | 32 | 11 | do | do | Do. |
| 161 to 165 | 33 | 11 | do | do | Do. |
| 166 to 170 | 34 | 11 | do | do | Do. |
| 171 to 175 | 35 | 11 | do | do | Do. |
| 176 to 180 | 36 | 14 | Nov. 4, 1918 | do | Do. |
| 181 to 185 | 37 | 14 | do | do | Do. |
| 186 to 190 | 38 | 14 | do | do | Do. |
| 191 to 195 | 39 | 14 | do | do | Do. |
| 196 to 200 | 40 | 14 | do | do | Do. |
| 201 to 205 | 41 | 14 | do | do | Do. |
| 206 to 210 | 42 | 14 | do | do | Do. |
| 211 to 215 | 43 | 14 | do | do | Do. |
| 216 to 220 | 44 | 14 | do | do | Do. |
| 221 to 225 | 45 | 14 | do | do | Do. |
| 226 to 230 | 46 | 14 | do | do | Do. |
| 231 to 235 | 47 | 14 | do | do | Do. |
| 236 to 240 | 48 | 14 | do | do | Do. |
| 241 to 245 | 49 | 14 | do | do | Do. |
| 246 to 250 | 50 | 14 | do | do | Do. |

TABLE II.—*Inoculations on young pummelo leaves from infusions of autoclaved soil in tubes made on consecutive days after inoculation with P. citri*

| Leaves No. | Infusion tube No. | Number of days after inoculation of tubes. | Date of inoculation from tubes to leaves. | Infections from 200 punctures. | Date of observation. |
|---|---|---|---|---|---|
| 1 to 5 | 1 | 2 | Oct. 23, 1918 | 100 per cent positive. | Nov. 2, 1918. |
| 6 to 10 | 2 | 2 | .....do..... | 99½ per cent positive. | Do. |
| 11 to 15 | 3 | 2 | .....do..... | 100 per cent positive. | Do. |
| 16 to 20 | 4 | 2 | .....do..... | .....do..... | Do. |
| 21 to 25 | 5 | 2 | .....do..... | .....do..... | Do. |
| 26 to 30 | 6 | 3 | Oct. 24, 1918 | .....do..... | Nov. 4, 1918. |
| 31 to 35 | 7 | 3 | .....do..... | .....do..... | Do. |
| 36 to 40 | 8 | 3 | .....do..... | .....do..... | Do. |
| 41 to 45 | 9 | 3 | .....do..... | .....do..... | Do. |
| 46 to 50 | 10 | 3 | .....do..... | .....do..... | Do. |
| 51 to 55 | 11 | 4 | Oct. 25, 1918 | .....do..... | Do. |
| 56 to 60 | 12 | 4 | .....do..... | .....do..... | Do. |
| 61 to 65 | 13 | 4 | .....do..... | .....do..... | Do. |
| 66 to 70 | 14 | 4 | .....do..... | .....do..... | Do. |
| 71 to 75 | 15 | 4 | .....do..... | .....do..... | Do. |
| 76 to 80 | 16 | 5 | Oct. 26, 1918 | .....do..... | Nov. 5, 1918. |
| 81 to 85 | 17 | 5 | .....do..... | .....do..... | Do. |
| 86 to 90 | 18 | 5 | .....do..... | .....do..... | Do. |
| 91 to 95 | 19 | 5 | .....do..... | .....do..... | Do. |
| 96 to 100 | 20 | 5 | .....do..... | .....do..... | Do. |
| 101 to 105 | 21 | 7 | Oct. 28, 1918 | .....do..... | Nov. 9, 1918. |
| 106 to 110 | 22 | 7 | .....do..... | .....do..... | Do. |
| 111 to 115 | 23 | 7 | .....do..... | .....do..... | Do. |
| 116 to 120 | 24 | 7 | .....do..... | .....do..... | Do. |
| 121 to 125 | 25 | 7 | .....do..... | .....do..... | Do. |
| 126 to 130 | 26 | 9 | Oct. 30, 1918 | .....do..... | Nov. 20, 1918. |
| 131 to 135 | 27 | 9 | .....do..... | .....do..... | Do. |
| 136 to 140 | 28 | 9 | .....do..... | .....do..... | Do. |
| 141 to 145 | 29 | 9 | .....do..... | .....do..... | Do. |
| 146 to 150 | 30 | 9 | .....do..... | .....do..... | Do. |
| 151 to 155 | 31 | 11 | Nov. 1, 1918 | .....do..... | Do. |
| 156 to 160 | 32 | 11 | .....do..... | .....do..... | Do. |
| 161 to 165 | 33 | 11 | .....do..... | .....do..... | Do. |
| 166 to 170 | 34 | 11 | .....do..... | .....do..... | Do. |
| 171 to 175 | 35 | 11 | .....do..... | .....do..... | Do. |
| 176 to 180 | 36 | 14 | Nov. 4, 1918 | .....do..... | Do. |
| 181 to 185 | 37 | 14 | .....do..... | .....do..... | Do. |
| 186 to 190 | 38 | 14 | .....do..... | .....do..... | Do. |
| 191 to 195 | 39 | 14 | .....do..... | 92½ per cent positive. | Do. |
| 196 to 200 | 40 | 14 | .....do..... | 100 per cent positive. | Do. |

SUMMARY OF EXPERIMENT I

Inoculations made upon young pummelo leaves from infusions made from autoclaved soil tubes inoculated with *P. citri* were uniformly 100 per cent positive or nearly so for 14 days following the inoculation of the soil tubes with *P. citri*. Inoculations from infusions from tubes of unsterilized soil in which *P. citri* was inoculated gave uniformly positive results on young pummelo trees during the first 3 days. Thereafter the percentages of positive results were low upon the fourth, fifth, and seventh days. On the ninth day there were but a few positive results

from a total of 1,000 punctures, and on the eleventh and fourteenth days 4,000 puncture inoculations from the infusions were entirely negative.

The evidence of this experiment therefore points to a gradual dying out of the canker organism in unsterilized soil, although in the sterilized soil the canker bacteria are very active.

## EXPERIMENT II

This experiment was undertaken to obtain all possible information upon the condition of *P. citri* in orchard soils.

Rain had fallen intermittently every day for 15 days. The Ellen grapefruit tree selected for this experiment showed 100 per cent of the leaves cankered, and in many cases the leaves had over 50 cankers apiece—that is to say, the tree was badly affected with canker and a drop of water could hardly fall to the ground from this tree without having been in contact with cankers.

During a violent shower, rain dripping from the leaves of this tree was collected in five culture tubes. These tubes were then carried to the isolation plots where citrus-canker had been excluded. From each tube of the rain water five young, actively growing grapefruit leaves were inoculated on upper and lower surfaces, each leaf being punctured at the same time with 40 needle stabs. The heavily cankered grapefruit tree was then cut down and removed, and all fallen leaves were removed from the ground. Soil from beneath the tree was then placed in five culture tubes, infusions were made and taken to the isolation plots, and five leaves were inoculated from each infusion. Forty punctures were made on each leaf, and both upper and lower surfaces were coated.

The twigs bearing the leaves inoculated with the infusion as well as those inoculated from the drip water were wrapped in paraffin paper with a piece of moistened cotton. The paraffin paper was then covered with opaque paper. A muslin tent was spread over the soil about the stump of the Ellen grapefruit tree after all fallen leaves had been removed. The tent prevented infected leaves from being blown upon the soil but allowed active play of rain and air as under normal conditions. The percentages of infection are given in Tables III and IV.

TABLE III.—*Inoculations on young pummelo leaves from rain water collected from the leaves of a badly cankered grapefruit tree*

| Leaves No. | Infusion tube No. | Date of inoculation on leaves. | Infections from 200 punctures. | Date of observation. |
|---|---|---|---|---|
| 1 to 5 | 1 | July 20, 1918 | 3 per cent positive | July 31, 1918. |
| 6 to 10 | 2 | .....do..... | All negative | Do. |
| 11 to 15 | 3 | .....do..... | 4½ per cent positive | Do. |
| 16 to 20 | 4 | .....do..... | 19½ per cent positive | Do. |
| 21 to 25 | 5 | .....do..... | 36½ per cent positive | Do. |

TABLE IV.—*Inoculations on young pummelo leaves made immediately after rain and on consecutive days following the rain from infusions of orchard soil from beneath a heavily infected grapefruit tree*

| Leaves No. | Infusion tube No. | Number of days between rain and inoculation. | Date of inoculation on leaves. | Infections from 200 punctures. | Date of observation. |
|---|---|---|---|---|---|
| 1 to 5 | 1 | Immediately after rain. | July 20, 1918 | All negative | July 31, 1918. |
| 6 to 10 | 2 | do | do | 8¾ per cent positive. | Do. |
| 11 to 15 | 3 | do | do | 4½ per cent positive. | Do. |
| 16 to 20 | 4 | do | do | 12½ per cent positive. | Do. |
| 21 to 25 | 5 | do | do | 1 per cent positive. | Do |
| 26 to 30 | 6 | 1 | July 21, 1918 | All negative | Do. |
| 31 to 35 | 7 | 1 | do | ½ of 1 per cent positive. | Do. |
| 36 to 40 | 8 | 1 | do | All negative | Do. |
| 41 to 45 | 9 | 1 | do | do | Do. |
| 46 to 50 | 10 | 1 | do | do | Do. |
| 51 to 55 | 11 | 2 | July 22, 1918 | ½ of 1 per cent positive. | Do. |
| 56 to 60 | 12 | 2 | do | All negative | Do. |
| 61 to 65 | 13 | 2 | do | do | Do. |
| 66 to 70 | 14 | 2 | do | do | Do. |
| 71 to 75 | 15 | 2 | do | do | Do. |
| 76 to 80 | 16 | 3 | July 23, 1918 | do | Aug. 2, 1918. |
| 81 to 85 | 17 | 3 | do | do | Do. |
| 86 to 90 | 18 | 3 | do | do | Do. |
| 91 to 95 | 19 | 3 | do | do | Do. |
| 96 to 100 | 20 | 3 | do | do | Do. |
| 101 to 105 | 21 | 4 | July 24, 1918 | do | Do. |
| 106 to 110 | 22 | 4 | do | do | Do. |
| 111 to 115 | 23 | 4 | do | do | Do. |
| 116 to 120 | 24 | 4 | do | do | Do. |
| 121 to 125 | 25 | 4 | do | do | Do. |
| 126 to 130 | 26 | 5 | July 25, 1918 | do | Aug. 3, 1918. |
| 131 to 135 | 27 | 5 | do | do | Do. |
| 136 to 140 | 28 | 5 | do | do | Do. |
| 141 to 145 | 29 | 5 | do | do | Do. |
| 146 to 150 | 30 | 5 | do | do | Do. |
| 151 to 155 | 31 | 7 | July 27, 1918 | do | Aug. 16, 1918. |
| 156 to 160 | 32 | 7 | do | do | Do. |
| 161 to 165 | 33 | 7 | do | do | Do. |
| 166 to 170 | 34 | 7 | do | do | Do. |
| 171 to 175 | 35 | 7 | do | do | Do. |
| 176 to 180 | 36 | 9 | July 29, 1918 | do | Do. |
| 181 to 185 | 37 | 9 | do | do | Do. |
| 186 to 190 | 38 | 9 | do | do | Do. |
| 191 to 195 | 39 | 9 | do | do | Do. |
| 196 to 200 | 40 | 9 | do | do | Do. |

SUMMARY OF EXPERIMENT II

Inoculations made from rain water collected from the foliage of a heavily infected grapefruit tree gave positive results upon young pummelo leaves. Inoculations made from infusions of the soil beneath such a heavily infected tree also gave positive results on pummelo leaves immediately following the rain. On the first day after the rain there

was one positive result and on the second day following the rain there was a positive result. Thereafter on the third, fourth, fifth, seventh, and ninth days the results were entirely negative. On these days a total of 125 leaves, or 5,000 punctures were inoculated with the soil infusion, and all remained negative.

The conclusion is reached, then, that although the canker organism was present immediately following the rain, in this case the citrus-canker organism has died out in the orchard soil.

## REPETITION OF EXPERIMENTS I AND II

The field data have been very extensive in support of the theory that the canker bacteria can exist and multiply in the soil. Since the idea has been so firmly held by growers and horticulturists that the canker organism does live in the soil, and because the data presented in the two preceding experiments indicate the contrary to be the case, both these experiments were repeated.

Experiment I was repeated, and the original results were entirely corroborated. It was found that *P. citri* was abundant in the unsterilized soil tubes during the first, second, and third days; during the fourth, fifth, seventh, and ninth days the inoculations were but very slightly positive; on the fourteenth day the organism showed three positive results from a total of 4,000 punctures. In the sterilized soil tubes *P. citri* gave almost uniformly 100 per cent results up to and including the fourteenth day.

Experiment II was carried through three times. The first trial has been reported here in detail. For the second and third trials the same methods were used. In a second trial the water dripping from the foliage of an infected grapefruit tree was shown to contain *P. citri* in a large percentage of cases. The soil beneath the tree, immediately following the rain, also gave a large number of positive results. On the second day after the rain and thereafter for four days inoculations from the soil beneath the same tree gave entirely negative results on the pummelo leaves. In a third trial no tests were made with the rain water on the leaves, but immediately following the rain a large number of positive results were obtained on pummelo leaves from inoculations with the soil infusion from beneath the cankered foliage. On the first day after the rain a few positive results were obtained from the soil infusions, but on the second day none of the inoculations resulted positively. On the third and eighth days there were again a few positive results, but on the tenth day 2,000 inoculations made from the soil upon the pummelo leaves remained entirely negative. These second and third trials entirely corroborate the experiments reported above and indicate that the citrus canker organism is entirely killed in orchard soils.

The tests of orchard soil were carried on at different seasons of the year and are representative of the conditions in the soil in very different

climatic periods in the Philippines. Two of the series of inoculations with orchard soil infusions were carried on in the middle of the rainy season when the soil was kept constantly wet by the rains. The third series of inoculations was carried on at the beginning of the dry season when the soil dried out and became dusty to a considerable extent. The attempt was made to secure the soil for each day's infusion at different depths. Soil was frequently taken from the surface and just as frequently from a depth of 10 inches. It is thought that the inoculations shown here were made from soil infusions which are entirely representative of the different conditions in the Lamao soils.

Inasmuch as the question of the existence or nonexistence of *P. citri* in the soil is an important point in canker control work, the following test was undertaken to corroborate further the preceding experiments.

### EXPERIMENT III

#### INOCULATED SOIL IN BOXES

Orchard soil was autoclaved twice, one hour each time at 45 pounds pressure. The soil was placed in thin layers on plates, so that the steam would penetrate easily. The autoclaved soil was placed in a seed-house flat which measured 18 by 24 by 5 inches. The soil was air-dried and was inoculated with 1,500 cc. of an infusion of *P. citri* in sterile water. This flat was then placed at a level with the soil and covered with cheesecloth to prevent animals from disturbing it. The flat received the full play of sun, wind, and rain and was exposed to the same conditions as exist beneath a tree in the orchard.

Another flat of the same size containing unsterilized soil was inoculated with an equal amount of an identical infusion. This flat was placed under identical conditions with the flat of sterilized soil but at several yards' distance to prevent distribution of the canker organism too easily; it was also covered with cheesecloth.

On the first day after inoculation, a small portion of the inoculated soil from the autoclaved flat was removed with a spatula to a clean dry-sterilized culture tube. To this about 10 cc. of tap water were added; the tube was shaken vigorously for several minutes; and the resulting infusion was spread upon the upper and lower surfaces of five actively growing pummelo leaves. Each leaf was then punctured 40 times with a new needle, and a new coating of the infusion was spread over the leaves and over the punctures. For this spreading of the infusion small cotton swabs such as are used for collecting diphtheria cocci from suspected cases were used. A new swab was used for each tube of infusion.

Five infusions were made each successive day from the flat of inoculated autoclaved soil. On each successive day five infusions were made in the same way from the unsterilized inoculated soil, and each of these was spread upon five actively growing leaves, each leaf being subsequently punctured 40 times.

Thus inoculations were made each day from 10 infusions of soil. These 10 infusions were identical in every way except that 5 were made from a flat of soil which had been inoculated with *P. citri* after being sterilized while the other 5 were made from a flat of soil which had been inoculated without being sterilized. Tables V and VI give the results of the inoculations.

TABLE V.—*Inoculation of young pummelo leaves from infusions of untreated soil in a box made on consecutive days after inoculation with P. citri. The inoculated soil was placed in the orchard to simulate field conditions*

| Leaves No. | Infusion tube No. | Number of days after inoculation into soil. | Date of inoculation on leaves. | Infections from 200 punctures. | Date of observation. |
|---|---|---|---|---|---|
| 1 to 5 | 1 | 1 | Oct. 23, 1918 | 77 per cent positive | Nov. 2, 1918. |
| 6 to 10 | 2 | 1 | do | 80 per cent positive | Do. |
| 11 to 15 | 3 | 1 | do | 87 per cent positive | Do. |
| 16 to 20 | 4 | 1 | do | 100 per cent positive | Do. |
| 21 to 25 | 5 | 1 | do | 53½ per cent positive | Do. |
| 26 to 30 | 6 | 2 | Oct. 24, 1918 | 22 per cent positive | Nov. 4, 1918. |
| 31 to 35 | 7 | 2 | do | 28 per cent positive | Do. |
| 36 to 40 | 8 | 2 | do | 54½ per cent positive | Do. |
| 41 to 45 | 9 | 2 | do | 78½ per cent positive | Do. |
| 46 to 50 | 10 | 2 | do | 73 per cent positive | Do. |
| 51 to 55 | 11 | 3 | Oct. 25, 1918 | 16½ per cent positive | Do. |
| 56 to 60 | 12 | 3 | do | 8½ per cent positive | Do. |
| 61 to 65 | 13 | 3 | do | 18½ per cent positive | Do. |
| 66 to 70 | 14 | 3 | do | 15½ per cent positive | Do. |
| 71 to 75 | 15 | 3 | do | 21 per cent positive | Do. |
| 76 to 80 | 16 | 4 | Oct. 26, 1918 | All negative | Nov. 5, 1918. |
| 81 to 85 | 17 | 4 | do | do | Do. |
| 86 to 90 | 18 | 4 | do | 1½ per cent positive | Do. |
| 91 to 95 | 19 | 4 | do | All negative | Do. |
| 96 to 100 | 20 | 4 | do | 6 per cent positive | Do. |
| 101 to 105 | 21 | 6 | Oct. 28, 1918 | All negative | Nov. 9, 1918. |
| 106 to 110 | 22 | 6 | do | do | Do. |
| 111 to 115 | 23 | 6 | do | do | Do. |
| 116 to 120 | 24 | 6 | do | do | Do. |
| 121 to 125 | 25 | 6 | do | do | Do. |
| 126 to 130 | 26 | 8 | Oct. 30, 1918 | do | Nov. 20, 1918. |
| 131 to 135 | 27 | 8 | do | do | Do. |
| 136 to 140 | 28 | 8 | do | do | Do. |
| 141 to 145 | 29 | 8 | do | do | Do. |
| 146 to 150 | 30 | 8 | do | do | Do. |
| 151 to 155 | 31 | 10 | Nov. 1, 1918 | do | Do. |
| 156 to 160 | 32 | 10 | do | do | Do. |
| 161 to 165 | 33 | 10 | do | do | Do. |
| 166 to 170 | 34 | 10 | do | do | Do. |
| 171 to 175 | 35 | 10 | do | do | Do. |
| 176 to 180[a] | 36 | 12 | | | |
| 181 to 185 | 37 | 12 | Nov. 3, 1918 | All negative | Nov. 20, 1918. |
| 186 to 190 | 38 | 12 | do | do | Do. |
| 191 to 195 | 39 | 12 | do | do | Do. |
| 196 to 200 | 40 | 12 | do | do | Do. |

[a] Leaves 176 to 180 were inoculated and then found to be already naturally infected at insect injuries. These leaves were therefore cut off Nov. 3, 1918, and were not carried in the experiment.

TABLE V.—*Inoculation of young pummelo leaves from infusions of untreated soil in a box made on consecutive days after inoculation with P. citri. The inoculated soil was placed in the orchard to simulate field conditions*—Continued

| Leaves No. | Infusion tube No. | Number of days after inoculation into soil. | Date of inoculation on leaves. | Infections from 200 punctures. | Date of observation. |
|---|---|---|---|---|---|
| 201 to 205 | 41 | 14 | Nov. 5, 1918 | All negative..... | Nov. 20, 1918. |
| 206 to 210 | 42 | 14 | .....do..... | .....do..... | Do. |
| 211 to 215 | 43 | 14 | .....do..... | .....do..... | Do. |
| 216 to 220 | 44 | 14 | .....do..... | .....do..... | Do. |
| 221 to 225 | 45 | 14 | .....do..... | .....do..... | Do. |
| 226 to 230 | 46 | 14 | .....do..... | .....do..... | Do. |
| 231 to 235 | 47 | 14 | .....do..... | .....do..... | Do. |
| 236 to 240 | 48 | 14 | .....do..... | .....do..... | Do. |
| 241 to 245 | 49 | 14 | .....do..... | .....do..... | Do. |
| 246 to 250 | 50 | 14 | .....do..... | .....do..... | Do. |
| 251 to 255 | 51 | 14 | .....do..... | .....do..... | Do. |
| 256 to 260 | 52 | 14 | .....do..... | .....do..... | Do. |
| 261 to 265 | 53 | 14 | .....do..... | .....do..... | Do. |
| 266 to 270 | 54 | 14 | .....do..... | .....do..... | Do. |
| 271 to 275 | 55 | 14 | .....do..... | .....do..... | Do. |

TABLE VI.—*Inoculations on young pummelo leaves from infusions of autoclaved soil in a box made on consecutive days after inoculation with P. citri. The inoculated soil was placed in the orchard to simulate field conditions*

| Leaves No. | Infusion tube No. | Number of days after inoculation into soil. | Date of inoculation on leaves. | Infections from 200 punctures. | Date of observation. |
|---|---|---|---|---|---|
| 1 to 5 | 1 | 1 | Oct. 23, 1918 | 96 per cent positive .. | Nov. 2, 1918. |
| 6 to 10 | 2 | 1 | .....do..... | .....do..... | Do. |
| 11 to 15 | 3 | 1 | .....do..... | 98 per cent positive .. | Do. |
| 16 to 20 | 4 | 1 | .....do..... | 95½ per cent positive . | Do. |
| 21 to 25 | 5 | 1 | .....do..... | 99 per cent positive .. | Do. |
| 26 to 30 | 6 | 2 | Oct. 24, 1918 | 100 per cent positive . | Oct. 30, 1918. |
| 31 to 35 | 7 | 2 | .....do..... | .....do..... | Do. |
| 36 to 40 | 8 | 2 | .....do..... | .....do..... | Do. |
| 41 to 45 | 9 | 2 | .....do..... | .....do..... | Nov. 4, 1918. |
| 46 to 50 | 10 | 2 | .....do..... | .....do..... | Do. |
| 51 to 55 | 11 | 3 | Oct. 25, 1918 | .....do..... | Do. |
| 56 to 60 | 12 | 3 | .....do..... | .....do..... | Do. |
| 61 to 65 | 13 | 3 | .....do..... | 99½ per cent positive . | Do. |
| 66 to 70 | 14 | 3 | .....do..... | 100 per cent positive .. | Do. |
| 71 to 75 | 15 | 3 | .....do..... | .....do..... | Do. |
| 76 to 80 | 16 | 4 | Oct. 26, 1918 | .....do..... | Nov. 5, 1918. |
| 81 to 85 | 17 | 4 | .....do..... | 96 per cent positive .. | Do. |
| 86 to 90 | 18 | 4 | .....do..... | 100 per cent positive . | Do. |
| 91 to 95 | 19 | 4 | .....do..... | .....do..... | Do. |
| 96 to 100 | 20 | 4 | .....do..... | .....do..... | Do. |
| 101 to 105 | 21 | 6 | Oct. 28, 1918 | 95½ per cent positive . | Nov. 9, 1918. |
| 106 to 110 | 22 | 6 | .....do..... | 100 per cent positive . | Do. |
| 111 to 115 | 23 | 6 | .....do..... | 99 per cent positive .. | Do. |
| 116 to 120 | 24 | 6 | .....do..... | 100 per cent positive . | Do. |
| 121 to 125 | 25 | 6 | .....do..... | 94½ per cent positive . | Do. |

TABLE VI.—*Inoculations on young pummelo leaves from infusions of autoclaved soil in a box made on consecutive days after inoculation with P. citri. The inoculated soil was placed in the orchard to simulate field conditions*—Continued

| Leaves No. | Infusion tube No. | Number of days after inoculation into soil. | Date of inoculation on leaves. | Infections from 200 punctures. | Date of observation. |
|---|---|---|---|---|---|
| 126 to 130 | 26 | 8 | Oct. 30, 1918 | 100 per cent positive | Nov. 9, 1918. |
| 131 to 135 | 27 | 8 | do | do | Do. |
| 136 to 140 | 28 | 8 | do | do | Do. |
| 141 to 145 | 29 | 8 | do | 76 per cent positive | Do. |
| 146 to 150 | 30 | 8 | do | 100 per cent positive | Do. |
| 151 to 155 | 31 | 10 | Nov. 1, 1918 | do | Nov. 20, 1918. |
| 156 to 160 | 32 | 10 | do | do | Do. |
| 161 to 165 | 33 | 10 | do | do | Do. |
| 166 to 170 | 34 | 10 | do | 99½ per cent positive | Do. |
| 171 to 175 | 35 | 10 | do | 100 per cent positive | Do. |
| 176 to 180 | 36 | 12 | Nov. 3, 1918 | do | Do. |
| 181 to 185 | 37 | 12 | do | 83½ per cent positive | Do. |
| 186 to 190 | 38 | 12 | do | 100 per cent positive | Do. |
| 191 to 195 | 39 | 12 | do | 93½ per cent positive | Do. |
| 196 to 200 | 40 | 12 | do | 96 per cent positive | Do. |
| 201 to 205 | 41 | 14 | Nov. 5, 1918 | 100 per cent positive | Do. |
| 206 to 210 | 42 | 14 | do | do | Do. |
| 211 to 215 | 45 | 14 | do | do | Do. |
| 216 to 220 | 44 | 14 | do | do | Do. |
| 221 to 225 | 45 | 14 | do | do | Do. |

SUMMARY OF EXPERIMENT III

It will be seen that inoculations made from the untreated soil were highly positive on the first day following inoculation with *P. citri*. On the second day there was a slight diminution of the positive results, and on the third day the percentages of positive results were very much lower. On the fourth day the larger part of the inoculations were entirely negative. On the sixth, eighth, tenth, twelfth, and fourteenth days following inoculation, all inoculations were entirely negative. That is, six days after the inoculation with a heavy infusion of *P. citri* in untreated soil, 170 leaves were inoculated, each with 40 punctures, or a total of 6,800 punctures; all remained negative. At the same time inoculations made on consecutive days following inoculations of autoclaved soil with *P. citri* were highly positive the first day, increased to almost uniformly 100 per cent positive results on the second day, and continued at 100 per cent for 14 days.

The full significance of this may perhaps be grasped more readily by a brief recapitulation. A dense infusion of virulent active canker organisms was heavily inoculated into a box of soil entirely untreated and but recently removed from the orchard. This box of inoculated but otherwise untreated soil was kept under orchard conditions during the experiment.

Six days after the inoculation with the heavy infusion no indications of the organism could be obtained from this soil. As a control upon the conditions a similar box of soil, alike in every detail except that it had been autoclaved, was inoculated; it showed the continuance of the canker bacteria throughout 14 days, and the bacteria were apparently as numerous on the fourteenth day as on the first.

These experimental results, as well as those with the tubed soils, indicate that the canker organism does not increase and multiply or live even a passive existence in the normal soil but is quickly killed out. Inasmuch, however, as it will live in soil from which all other organisms are excluded, there is indication that in unsterilized soil the activities of the normal soil organisms are antagonistic to the existence of *P. citri*.

The following results obtained by a different experimental procedure still further corroborate the previous conclusions.

EXPERIMENT IV

This experiment was conducted to show the persistence or absence of the canker bacteria by growing susceptible plants in inoculated soils.

Ten bamboo pots were autoclaved and subsequently filled with soil twice autoclaved. These soil pots were then heavily inoculated with a dense infusion of *P. citri* in sterile water. On the same day 30 bamboo pots filled with unsterilized soil were inoculated with the canker organism from similar infusions.

Pots 1 to 5 of sterilized, inoculated soil were immediately planted each with 10 seeds from *Citrus trifoliata* fruits; pots 11 to 20 of unsterilized, inoculated soil were also immediately planted each with 10 seeds of *C. trifoliata*. After an interval of 5 days 10 more pots of unsterilized, inoculated soil were planted each with 10 seeds; and after an interval of 10 days 10 pots of unsterilized soil and 5 more pots of sterilized soil were planted, each pot with 10 *C. trifoliata* seeds.

It was the intention, of course, that the *Citrus trifoliata* seedlings resulting would be very susceptible and in growing through the inoculated soil would become infected if the canker organism still remained alive within the soil.

Running parallel with these series of inoculated soil pots, a series of orchard soil pots was operated as follows: Ten pots were filled with soil taken from beneath a heavily infected grapefruit tree, immediately following a rain, and each pot was planted with *Citrus trifoliata* seeds. The tree was cut down and all sources of infection were removed from the soil; then 10 days later 10 more pots were filled with the same soil and similarly planted.

All pots, those containing orchard soil naturally infected and those artificially inoculated, were covered with cheesecloth after planting to prevent the ingress and egress of insects which might spread infection.

The two series of pots were kept separated in the dense tropical woods at Lamao. The inoculation and planting data with results are given in Tables VII and VIII.

TABLE VII.—*Results of sprouting seeds and growing young plants of Citrus trifoliata in pots of soil artificially inoculated with canker bacteria*

| Pot No. | Treatment of soil. | Date of inoculation. | Date of planting. | Results. | Date of examination. |
|---|---|---|---|---|---|
| 1 | Sterilized | Oct. 22, 1918 | Oct. 22, 1918 | No trees. | Jan. 16, 1919. |
| 2 to 5 | do. | do. | do. | 9 trees, no infections. | Do. |
| 6 to 10 | do. | do. | Nov. 1, 1918 | 10 trees, no infections. | Do. |
| 11 to 20 | Unsterilized | do. | Oct. 22, 1918 | 31 trees, no infections. | Do. |
| 31 to 33 | do. | do. | Oct. 27, 1918 | 7 trees, no infections. | Do. |
| 34 | do. | do. | do. | No trees. | Do. |
| 35 to 40 | do. | do. | do. | 19 trees, no infections. | Do. |
| 51 to 58 | do. | do. | Nov. 1, 1918 | do. | Do. |
| 59 to 61 | do. | do. | do. | No trees. | Do. |

TABLE VIII.—*Results of sprouting seeds and growing young plants of Citrus trifoliata in pots of soil naturally infected with canker bacteria in the orchard*

| Pot No. | Condition of soil. | Length of time after rain. | Date of planting. | Results. | Date of examination. |
|---|---|---|---|---|---|
| 21 to 22 | Naturally infected. | Immediately after rain. | Oct. 23, 1918 | No trees. | Jan. 16, 1919. |
| 23 to 30 | do. | do. | do. | 15 trees, no infections. | Do. |
| 41 to 42 | do. | 5 days. | Oct. 28, 1918 | No trees. | Do. |
| 43 to 50 | do. | do. | do. | 18 trees, no infections. | Do. |
| 62 to 66 | do. | 13 days. | Nov. 5, 1918 | No trees. | Do. |
| 67 to 69 | do. | do. | do. | 4 trees, no infections. | Do. |
| 70 | do. | do. | do. | No trees. | Do. |
| 71 | do. | do. | do. | 1 tree, no infections. | Do. |

SUMMARY OF EXPERIMENT IV

One hundred and thirty-three seedling *Citrus trifoliata* trees were sprouted in soil pots. These pots had been inoculated with the canker organism, either by artificial or natural means, from 35 to 40 days previous to the sprouting of the seeds. None of the seedlings at any time showed canker, although they were kept for 45 days after they appeared above the ground. The seedlings were from seed taken from heavily infected *C. trifoliata* fruits on badly infected *C. trifoliata* trees; there can be no doubt as to the general susceptibility of the stock. The strain of the organism used in inoculating the soil was the same as that which produced lesions

upon the lansones (*Lansium domesticum*), and there can be no question as to its virulence. The temperatures and humidity were at all times favorable for the development of canker.

Theoretically, criticism of the results of this experiment might be raised, since none of the trees, even the controls in sterilized inoculated soil, showed canker. Practically, however, there is a very good explanation. The seeds did not begin to sprout and the young shoots to push through the soil until the first week in December—that is, 35 days after soil was noculated. During this time the sterilized soil pots were exposed in the Lamao woods, protected only from contamination by coarse cheesecloth. Under these conditions it could be expected that a few weeks after being placed in the woods the soil in the pots would be well inoculated with the ordinary soil flora and the canker organism would then be killed out. Another explanation might be that the normal young seedlings of *Citrus trifoliata* are possibly resistant to citrus canker infection, in which event the value of this method of testing for soil infection would be lessened.

## SUMMARY OF RESULTS OF EXPERIMENTS

It has been shown in two separate experiments that *P. citri* lives and may even increase in culture tubes of sterilized soil throughout a period of 14 days or more. On the other hand, tubes of identical soil, handled in an identical manner with the exception of not being autoclaved, showed the canker organism to be entirely killed out within a period of 6 days.

In three similar experiments, representing two distinct seasonal periods, it was shown that the canker organism can be found in the soil beneath a heavily infected tree on the day immediately following the rain and on the second and third days following. Thereafter there is no indication of the canker organism in the soil.

In another experiment a box of soil was autoclaved and then inoculated with *P. citri*. This box, placed in the orchard to simulate field conditions, showed no decrease in the activity of the canker organism during a period of 14 days after inoculation. A box of similar soil, treated in an identical manner with the exception of not being autoclaved, showed the canker organism to be entirely killed out within a period of 6 days.

In the last experiment seeds were planted in nonsterile soil which had been inoculated with a heavy infusion of *P. citri*. The seeds which germinated and pushed through the soil 40 days after inoculation never showed any sign of canker although they were kept for 45 days after their appearance above the soil.

The results of each series of experiments point to the dying out of the canker organism in untreated soils. The indication is that the normal soil organisms are antagonistic in some way to the existence of *P. citri* in the soil

The soil at Lamao is a sandy loam and seems to be of alluvial origin. There is little or no indication of decaying organic matter in the soil

and there is no reason to base a supposition for unusual bacterial activity on such grounds. The soil used in the experiments was taken from directly beneath trees of the Ellen grapefruit variety and was plowed, cultivated, and hoed according to usual orchard practices. The treatment of the soil differed very little from that usually practiced in the United States.

## APPLICATION OF RESULTS

The writer would prefer that any applications of these findings be made by the field men, who are in the best position to judge the merits of different methods in eradication work. The following suggestion might be made, however, from a theoretical viewpoint.

It is frequently stated that canker is carried from orchard to orchard upon muddy feet or in the earth upon farm implements. These statements appear to be based upon a wrong conception of the character of the canker organism, and it would seem probable that the disease bacteria are carried upon dry portions of clothing and implements rather than in the earth. These experiments should therefore serve not to decrease the vigilance of quarantine measures but to increase the precautions to eliminate all sources for reinfection and dissemination of canker; for inasmuch as these experiments indicate that the canker organism does not live in the soil, field data which seem to indicate that *P. citri* is a soil inhabitant must be explained as indications of a source of reinfection overlooked or of a careless transfer of the organisms by farm animals or man.

## SOME POSSIBLE SOURCES FOR REINFECTION BY THE CANKER ORGANISM

It has been demonstrated by Peltier and Neal [1] that the canker organism may overwinter in the bark tissue of citrus trees. The following observations may supplement their findings as to the means of overwintering or survival.

In the Philippine Islands lesions very much resembling those of citrus-canker were observed upon the mature wood of grapefruit trees (*Citrus maxima*) and lime (*C. aurantifolia*). These lesions were of a slightly lighter brown color than the normal bark and consisted of eruptions of tissue very similar to cankers upon leaves. Examinations of frozen sections of such eruptions revealed the typical structure of citrus-canker and the masses of bacteria distributed as in leaf cankers. *P. citri* was subsequently isolated from these lesions. Photographs (Pl. 36) show these mature wood cankers better than a description. The mature wood cankers were also observed upon navel orange trees (*C. sinensis*) in orchards in Japan.

Close examination has revealed that these mature wood cankers are by no means uncommon on lime, grapefruit, and sweet orange trees;

[1] PELTIER, George L., and NEAL, David C. OVERWINTERING OF THE CITRUS-CANKER ORGANISM IN THE BARK TISSUE OF HARDY CITRUS HYBRIDS. *In* Jour. Agr. Research, v. 14, no. 11, p. 523–524, pl. 58. 1918.

and their manner of occurrence indicates that wounds are not necessary for infection. They are commonly to be found upon branches as large as 2 or even 3 inches in diameter, the wood of which has entirely hardened and matured. One case has been observed of such cankers on the trunk of a lime tree 6 inches in diameter. Such cankers have never been seen on species other than the lime, the sweet orange, and the grapefruit. Cankers occurring in this way do not cause the killing of the limbs or branches, their seriousness consisting chiefly in affording constant sources for reinfection of foliage and fruit. Such cankers are also easily overlooked, inasmuch as they are small and of the same color as the normal bark.

The presence of such cankers suggested that cankers might also occur upon the roots of trees. Inoculations were therefore attempted upon roots with *P. citri* by means of needle punctures. The inoculations reacted slowly, but in 30 days examination showed that some of the punctures were undoubtedly positive. Control punctures with tap water were negative. The inoculations were then made repeatedly. The best series of results is selected here for presentation in Table IX. A photograph (Pl. 37, A) also shows some of these results. Mature trees, actively growing and thrifty, were selected for inoculation.

TABLE IX.—*Results of inoculations with P. citri by means of needle punctures into roots of Citrus sinensis*

| Inoculation No. | Inoculum. | Diameter of root. | Date of inoculation. | Result. | Date of examination. |
|---|---|---|---|---|---|
| | | *Mm.* | | | |
| 119 | *P. citri* culture | 4 | Dec. 5, 1917 | Positive | Feb. 15, 1918. |
| 120 | do | 4 | do | do | Do. |
| 121 | do | 4 | do | do | Do. |
| 122 | do | 4 | do | do | Do. |
| 123 | do | 4 | do | do | Do. |
| 124 | do | 4 | do | do | Do. |
| 125 | do | 4 | do | do | Do. |
| 126 | do | 4 | do | do | Do. |
| 127 | do | 4 | do | do | Do. |
| 128 | do | 4 | do | do | Do. |
| 129 | Tap water | 4 | do | Negative | Do. |
| 130 | do | 4 | do | do | Do. |
| 131 | do | 4 | do | do | Do. |
| 132 | do | 4 | do | do | Do. |
| 133 | do | 4 | do | do | Do. |
| 134 | do | 8 | do | do | Do. |
| 135 | do | 8 | do | Swelling (no eruption). | Do. |
| 136 | do | 8 | do | Lost | Do. |
| 137 | do | 8 | do | Negative | Do. |
| 138 | do | 8 | do | do | Do. |

The inoculations were made with a needle, and the punctures were covered with moist cotton and wrapped in paraffin paper, then in opaque paper, and covered with earth.

From such positive results of inoculations *P. citri* was several times reisolated; and such cultures reinoculated on leaves of *Citrus maxima* gave positive results. There is therefore a possibility, considered however to be small, that the canker organism is carried on the roots.

In digging in the soil beneath citrus trees in the Philippines, leaves have been uncovered upon which cankers were found. These leaves were skeletonized by the soil organisms, the lignified tissues apparently resisting the action of the soil organisms while the cellulose parts of the leaf blade had entirely disappeared. Canker lesions upon such buried leaves also seem to resist the dissolving action of the soil bacteria. Photographs (Pl. 37, B) show the persistence of cankers upon such buried skeletonized leaves. Whether cankered leaves which have been buried and subsequently uncovered may possibly furnish another means of carrying the canker organism over in spite of control measures is a question that deserves special experimental investigation.

In Florida there have been many cases of seemingly thorough eradication of the disease followed by a new outbreak, even after considerable periods of inactivity. Such outbreaks at the time have been the cause for considerable conjecture and speculation. It is possible that the results presented here may point to hitherto overlooked sources of new infection occurring after a period of latency.

## SUMMARY

(1) Experimental evidence is given to show that *P. citri* disappears from unsterilized soil in tubes and boxes usually within six days after they are inoculated. *P. citri* inoculated in sterilized soil increases and multiplies. Since the main difference in this latter case is the exclusion of the normal soil organisms, the disappearance of *P. citri* seems to be ascribable to the antagonistic effect of such soil inhabitants.

(2) In soil under orchard conditions, the canker organism is found to disappear even more rapidly than in the soil confined in boxes or culture tubes.

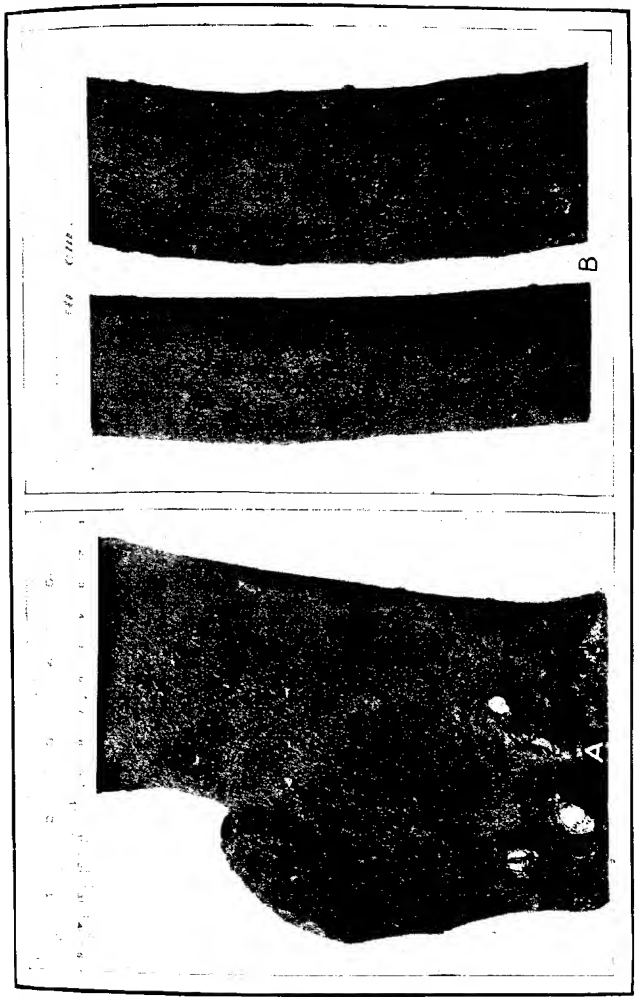
(3) Seeds were planted in pots of soil naturally infected with the canker organism and in pots of soil artificially inoculated. The seedlings came through the soil and developed normally without any canker, thus corroborating the conclusion that the canker bacteria are killed out in normal soils.

(4) Cankers upon mature wood of citrus trees and positive inoculations upon the roots of citrus trees are shown. Cankers upon buried leaves and mature wood and roots as possible sources of holding over the canker organism are suggested.

PLATE 36

A.—Citrus-cankers on mature wood of trunk of *Citrus aurantifolia*. Slightly reduced.

B.—Citrus-cankers on mature wood of branches of *Citrus aurantifolia*. Natural size.


B
A

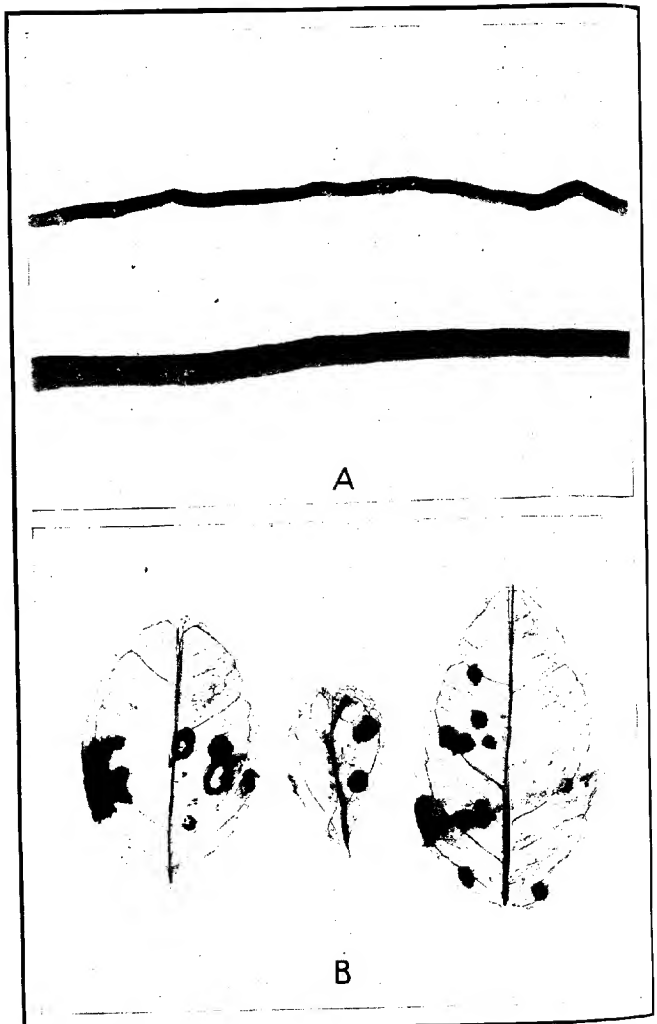

A
B

PLATE 37

A.—Results of inoculations with *Pseudomonas citri* upon roots of sweet orange (*Citrus sinensis*). Natural size.

B.—Skeletonized leaves of Ellen grapefruit recovered from buried soil. The leaf blade is entirely decomposed, leaving only the lignified veins and the cankered tissue. Natural size.

# DECLINE OF PSEUDOMONAS CITRI IN THE SOIL

By H. R. FULTON

*Pathologist, Fruit-Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

This investigation was undertaken primarily to determine whether or not the citrus-canker organism, *Pseudomonas citri* Hasse, is capable of persisting in the soil to such an extent as to make the soil an important medium in holding over or disseminating the organism.

## EXPERIMENTAL METHODS

The work has been conducted in an isolated greenhouse near Washington, D. C. During the tests the soils were kept in ordinary 4- or 6-inch earthenware flower pots in duplicate, triplicate, or quadruplicate sets for each test. For the original inoculation of the soil it was found most satisfactory to use washings from potato cylinder cultures 2 to 10 days old. One such culture tube diluted with 200 cc. of water would give heavy inoculation in a 4-inch pot. The bacterial suspension was well mixed with the upper 3 inches of the soil, and samples were taken from this portion from at least three different points.

Because of the preponderance of more rapidly growing soil organisms, ordinary plating methods are inadequate for determining the abundance of *P. citri* in soil samples, and recourse was had to inoculation of punctured mature grapefruit leaves with graded dilutions of washings from the soil to be tested. The procedure was as follows: A sample of about 20 gm. of the soil was removed with a sterile spoon to a sterile Petri dish, and enough sterile distilled water was added to give an excess of about 10 cc. beyond saturation. This was well stirred, and 1 cc. of the soil solution was transferred to another Petri dish in which 9 cc. of sterile distilled water had been previously placed. The first washing described above is referred to as the 1/1 dilution in this paper, and the second as the 1/10 dilution. In a similar way dilutions of 1/100, 1/1,000 or beyond were made from the original soil wash water. Small wefts of sterile absorbent cotton were placed in each dish, one for each leaf to be inoculated. The grapefruit seedlings used were grown in 2, 2½, or 3 inch pots. They averaged 6 or 8 inches in height and had as a rule 8 to 12 leaves. Usually 5 leaves per plant were used for inoculation, and each leaf was punctured at 100 points. A simple device for making these punctures rapidly and accurately was improvised by inserting 10 sewing needles through a small cork stopper. This "punch" was readily sterilized by flaming the needles, was convenient to handle, made the punctures in a uniform group pattern, and thus contributed materially to the rapidity

Journal of Agricultural Research,
Washington, D. C.
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Vol. XIX, No. 5
June 1, 1920
Key No. G-193

and accuracy of the work. A leaf was inoculated by wiping both under and upper surface of the freshly punctured portion with a cotton swab from a dilution dish, the swab being finally left on the upper surface. The inoculated plant was wrapped in paraffin paper, which served to retain moisture and to prevent accidental contamination from outside sources. Other plants were inoculated with pure cultures of *P. citri* as controls, and others were set up with the swabs merely wet with sterile water. The series were held at least a week in glass inoculation cases where conditions were near the optimum for canker development; later they were removed to the greenhouse benches. The first observations and records were made as a rule two to four weeks after inoculation. Final records were deferred until four to eight weeks after inoculation in order to insure the detection of any unusually slow development of infection such as occurred when the inoculum contained only a few organisms. The records show that between 90 and 95 per cent of the infections were apparent at the first observation and that no material increase was secured by holding beyond the second observation.

Variations of this method were tried out during its evolutionary development and to some extent in routine work as special considerations seemed to warrant. In many of the earlier series absorbent cotton wicks from small bottles of sterile water were placed in contact with the inoculation swabs on the leaves. This precaution to secure a prolonged moist condition proved to be unnecessary. An inoculum of mud paste, made by adding only a little water to the soil sample and applied with a backing of cloth or cotton as a sort of poultice over the punctured area, gave distinctly fewer infections than the soil solution in much greater dilutions. In cases where a large quantity of liquid inoculum could be prepared, a very effective method of inoculation was to dip the whole top of the test plant with its punctured leaves, keeping it submerged for an hour or longer, with several shakings during the period. In a few instances the test plants were so placed that the punctured leaves remained buried in the soil of the pots for a day or two. Tests were made of placing the plants under an air exhaust after soil water had been applied to their leaf surfaces. The soil solution was centrifuged to concentrate the canker organisms when they were very few, but this was without definitely satisfactory results. Still another method [1] employed was to atomize the leaves with sterile water, sift over them the rather dry soil to be tested, and keep the leaf surfaces moist for several days by holding the plants in a moist chamber and by repeatedly atomizing them with sterile water.

It was not apparent that any of the modifications of testing procedure could be relied upon to give a larger percentage of infections than the standard method, or to show the presence of *P. citri* when the standard method failed to give positive results.

[1] This method was first used by Miss Clara H. Hasse, of this office.

SENSITIVENESS OF DILUTION METHOD OF TESTING

In various tests involving several thousand plants, the standard testing method, which employs graded dilutions of the soil washing for inoculation on punctured grapefruit leaves, has proved reasonably sensitive in detecting the presence of viable *P. citri* in the soil. It is satisfactory for securing a rather definite idea of the relative numbers of this organism at the various times of sampling.

To test the efficiency of the method, dilutions in decimal series were made from a loopful of potato cylinder culture of *P. citri* distributed in the requisite number of cubic centimeters of sterile distilled water and were carried well beyond the vanishing point. One-cc. portions from each dilution were plated in beef agar for *P. citri* counts. Cotton swabs were dipped in the remainder of each dilution and applied to grapefruit leaves having 100 punctures each. Measurement showed these swabs to carry an average of 0.7 cc. of the liquid. The results of two independent tests are given in Table I.

TABLE I.—*Comparison between number of infections on grapefruit leaves and counts on poured plates, using graded dilutions of P. citri*

| | 1/10,000 | 1/100,000 | 1/1,000,000 | 1/10,000,000 | 1/100,000,000 | 1/1,000,000,000 | 1/10,000,000,000 |
|---|---|---|---|---|---|---|---|
| **TEST A** | | | | | | | |
| Average number of infections, 20 leaves tested | 76 | 14 | 2 | 0.2 | 0 | 0 | 0 |
| Average count for 1 cc. inoculum, 5 plates | 22,300 | 2,500 | 300 | 32 | 1 | 0 | 0 |
| Average number of organisms applied per leaf | 15,600 | 1,750 | 210 | 22 | ..... | ..... | ..... |
| Average number of organisms per infection | 205 | 125 | 105 | 110 | ..... | ..... | ..... |
| **TEST B** | | | | | | | |
| Average number of infections, 16 leaves tested | 69 | 9 | 1.4 | 0.13 | 0 | 0 | 0 |
| Average count for 1 cc. inoculum, 6 plates | 25,000 | 2,500 | 277 | 28 | 2.3 | 0.5 | 0.2 |
| Average number of organisms applied per leaf | 17,500 | 1,750 | 194 | 20 | ..... | ..... | ..... |
| Average number of organisms per infection | 253 | 194 | 139 | 154 | ..... | ..... | ..... |

The method apparently gives evidence of something like 30 organisms per cubic centimeter of inoculum, provided as many as 20 test leaves with 100 punctures each are used. The upper limit of sensitiveness would evidently be reached when numbers of bacteria are sufficient to infect practically all punctures, and diminution of sensitiveness would appear earlier. The ratio between infections per leaf and bacteria

applied is seen to be fairly uniform for the more critical lower ranges in both tests and lies between 1 to 100 and 1 to 200.

On the mature grapefruit leaves used for the tests there was practically no infection except at the freshly made punctures, and the counts are therefore free from errors that might have arisen from secondary spread if the unwounded tissue had been highly susceptible.

Two things are involved in the causation of infection by very dilute inoculum: (1) the chance for the organisms to reach the punctures and (2) the average number of organisms required to initiate infection successfully at a given point. In using cotton swabs, a considerable proportion of the organisms would be held at a distance from the leaf surface, and of those actually in the surface moisture film many would be at relatively great distances from punctures. On the other hand, motility of the organism and the possibility of rapid numerical increase by division would increase the chance for infection. As to the minimum number of *P. citri* organisms necessary to set up infection on reaching a given puncture, further careful experiments must be conducted before an opinion can be ventured.

## PERSISTENCE IN VARIOUS TYPES OF SOIL

To secure as great diversity as possible with types of soil conveniently at hand the following kinds were selected: (1) stiff clay subsoil thrown out from an excavation several months previously; (2) leaf mold screened from ground surface in forest; (3) rotting compost of sod and manure thoroughly decayed; (4) garden soil, a clay loam of moderate fertility. Four-inch pots were used in duplicate for each soil type. The inoculum for each pot amounted to 10 cc. of a 2-day beef bouillon culture of *P. citri* mixed with about one-fifth of the washing from a 6-day potato cylinder culture, the whole being diluted to 100 cc. and evenly mixed with the upper 3 inches of soil in the pot. Inoculations were made on August 14, 1918. The pots were held in the greenhouse shaded from direct sunlight, and were given ordinary watering. Each percentage in Table II is based on the number of infections developed in 17 days in a total of 600 leaf punctures and represents the average of duplicate pots of each soil type.

TABLE II.—*Percentages of infection on grapefruit leaves inoculated with graded dilutions of solutions from four types of soil made at various intervals after the soil had been inoculated with P. citri*

| Number of days between inoculation and sampling. | Clay subsoil. | | | | Leaf mold. | | | | Compost. | | | | Garden soil. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1/1 | 1/10 | 1/100 | 1/1,000 | 1/1 | 1/10 | 1/100 | 1/1,000 | 1/1 | 1/10 | 1/100 | 1/1,000 | 1/1 | 1/10 | 1/100 | 1/1,000 |
| 0 | 28 | 52 | 15 | 0.3 | 55 | 63 | 13 | 2 | 35 | 16 | 8.3 | 3 | 60 | 33 | 32 | 2.3 |
| 2 | 9.2 | 11 | 0.8 | 0 | 17 | 8.7 | 0.8 | 0 | 11 | 22 | 57 | 4.7 | 60 | 33 | 28 | 14 |
| 5 | .3 | 0 | 0 | 0 | 0.5 | .2 | 0 | 0 | 38 | 32 | 5.2 | .7 | 57 | 38 | 6.7 | .5 |
| 9 | 0 | 0 | 0 | 0 | .2 | 0 | 0 | 0 | 8.0 | 0.2 | .2 | 0 | 3.5 | 0.7 | 0 | .2 |
| 14 | 0 | 0 | .... | .... | 0 | 0 | .... | .... | 0 | 0 | .... | .... | 0 | 0 | .... | .... |

*P. citri* evidently decreased very rapidly in all these soils and apparently reached the vanishing point in all in less than 14 days. The rate of decrease was most rapid for the clay subsoil, slightly less so for the leaf mold, and distinctly slower for the compost and garden soil.

A second test was begun September 7, 1918, with new lots of soil from the same sources with the addition of well-washed sand from a creek bed, and a mixture of equal parts of the leaf mold and garden soil used in the earlier experiment. The initial inoculation was about 50 per cent heavier than in the preceding series. In order of rapidity of decrease clay subsoil proved again to be first, followed by leaf mold, sand, compost, garden soil, and mixture of leaf mold and compost. At the termination of this test, 14 days after inoculation, the red clay was the only one giving negative results; and the percentages for the leaf mold, compost, and garden soil were approximately those given for the ninth day in Table I. This longer persistence in the second test may reasonably be attributed to the higher initial inoculation of the soil.

In other experiments the following citrus soils from Florida were used: (1) from Orlando, intermediate between high and low pine soil types, unusually rich in humus; (2) similar to (1) but naturally poor; (3) similar to (2) but from a poorly drained spot; (4) from Bradentown, low pine land of low fertility; (5) from Bradentown, typical muck, extremely rich in humus; (6) from Winter Park, high hammock type; (7) from Winter Park, low hammock type. The samples, as a rule, reached the laboratory and were set up before becoming dry. The usual dilutions to 1/1,000 were run, but for brevity the percentages from the 1/1 dilution only are given in Table III. At the higher dilutions the commencement of decline was evident at the second sampling for all types, whereas this decline is not evident from the 1/1 figures of the table until the fifth or sixth day. The tests were made during September and October, 1918, in three distinct series, as indicated in the table. The second and third were conducted by Miss Clara H. Hasse, of this office, through whose courtesy the results have been furnished for this publication. The percentages are based on infection development from 600 punctures.

TABLE III.—*Percentages of infection on grapefruit leaves inoculated with 1/1 soil solution at various intervals after the soil had been inoculated with P. citri*

| Series 1. | | | | Series 2. | | | Series 3. | | |
|---|---|---|---|---|---|---|---|---|---|
| Number of days between inoculation and sampling. | Soil 1. | Soil 2. | Soil 3. | Number of days between inoculation and sampling. | Soil 4. | Soil 5. | Number of days between inoculation and sampling. | Soil 6. | Soil 7. |
| 0..... | 88 | 93 | 80 | 0..... | 31 | 62 | 0..... | 92 | 83 |
| 2..... | 90 | 93 | 88 | 3..... | 61 | 98 | ..... | ..... | ..... |
| 5..... | 45 | 50 | 33 | 6..... | 48 | 60 | 6..... | 34 | 41 |
| 9..... | 6.3 | 5.3 | 0.7 | 10..... | 2.2 | 2.8 | 10..... | 6.8 | 4.5 |
| 14..... | 2.5 | 0 | .5 | 16..... | .3 | 3.7 | 15..... | 4.8 | 1.5 |
| ..... | ..... | ..... | ..... | 48..... | 1.0 | 7.1 | 22..... | .2 | .5 |
| ..... | ..... | ..... | ..... | 56..... | 0 | 4.2 | 52..... | .2 | 0 |

There is a marked decline preceding the tenth day in all of the soils of Table III. But scattering infections are apparent over a much longer period, and no one of these soils could be safely declared free of *P. citri* at the times of discontinuance of the respective tests. It is a fact that regular watering of the pots was overlooked during the latter part of the longer tests, and the dry condition probably contributed to the long persistence. Special evidence on this point is given later in this paper.

Florida soils were also used in a number of other special tests, accounts of which follow throughout this paper.

Samples of soil from citrus plantings at Biloxi and Big Point, Miss., were artificially inoculated and tested at 6-day intervals for persistence of *P. citri*. The results were negative on the twelfth day and afterwards.

## INFLUENCE OF DEGREE OF INITIAL SOIL INOCULATION

A series was set up September 16, 1918, using three degrees of inoculum, one five times and another one-fifth the usual medium degree. Unfortunately this series was discontinued on the twelfth day, just when the decline from the heavy inoculation was beginning to be pronounced. A second test was begun October 20, 1919. Greenhouse potting soil was used. The medium inoculation consisted of 0.4 of the washings from a potato cylinder culture for each of the duplicate pots. The heavy inoculation was 10 times this, and the light inoculation one-tenth. The pots were kept in the greenhouse, were shaded, and were given ordinary watering. Each percentage given in Table IV is based on 2,000 inoculated punctures.

TABLE IV.—*Percentages of infection on grapefruit leaves inoculated with graded dilutions of soil solution at various intervals after the soil had been inoculated with P. citri in different degrees*

| Number of days between inoculation and sampling. | Heavy inoculation. | | | | Medium inoculation. | | | | Light inoculation. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1/1 | 1/10 | 1/100 | 1/1,000 | 1/1 | 1/10 | 1/100 | 1/1,000 | 1/1 | 1/10 | 1/100 | 1/1,000 |
| 0 | 62 | 23 | 5.8 | 0 | 9.8 | 4.8 | 0.3 | 0.6 | 6.0 | 0 | 0 | 0 |
| 2 | 33 | 30 | 5.5 | 1.0 | 9.1 | 3.0 | .4 | .2 | 1.0 | 0.1 | 0 | 0.05 |
| 4 | 11 | 3.5 | 1.6 | .4 | 1.4 | 0 | .05 | 0 | 0 | .05 | 0 | 0 |
| 7 | 4.6 | 1.0 | .4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | .3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | .05 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | .3 | .05 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ..... | 0 | 0 | 0 | ..... |
| 23 | .2 | 0 | 0 | ..... | 0 | 0 | ..... | ..... | 0 | 0 | ..... | ..... |
| 30 | 0 | ..... | ..... | ..... | 0 | ..... | ..... | ..... | 0 | ..... | ..... | ..... |

It is not understood why all the initial soil inoculations in this series turned out to be so far below the expected degree. What was intended for heavy soil inoculation ran considerably below that ordinarily used in other experi-

mental tests. At the same time it is probably as high as would be encountered in citrus plantings under infected trees; and the whole series may be regarded as representing high, medium, and low degrees of soil infection under natural conditions.

The differences are apparently not so much in rate of decline as in time required to reach the zero level from the different initial levels of inoculation.

## INFLUENCE OF SOIL TEMPERATURE ON PERSISTENCE

The test for low temperature effect, series 1, which is reported in Table V, was made by exposing the inoculated potting soil to outdoor temperatures, beginning October 11, 1918. During the test the minimum daily readings ranged from 60° to 23° F., and the maximum daily readings from 83 to 58°. For moderate temperatures, exposure was made in the greenhouse, with daily means averaging about 15° higher than outside. Series 2 was begun October 20, 1919, using an incubator at 95° for the high range and the greenhouse for the moderate. The actual soil temperatures 2 inches below the surface were taken, the high temperature test ranging from 86° to 90° and the moderate from 68° to 72°. Percentages for series 1 are based on 600 inoculated punctures, and for series 2 on 2,000.

TABLE V.—*Percentages of infection on grapefruit leaves inoculated with graded dilutions of soil solutions at various intervals after the soil had been inoculated with P. citri and had been held at different temperatures*

| Series 1. | | | | | | | | | Series 2. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Days between inoculation and sampling. | Moderate temperature. | | | | Low temperature. | | | | Days between inoculation and sampling. | Moderate temperature. | | | | High temperature. | | | |
| | 1/1 | 1/10 | 1/100 | 1/1,000 | 1/1 | 1/10 | 1/100 | 1/1,000 | | 1/1 | 1/10 | 1/100 | 1/1,000 | 1/1 | 1/10 | 1/100 | 1/1,000 |
| 0 | .... | .... | 66 | 7.2 | .... | .... | 53 | 12 | 0 | 9.8 | 4.8 | 0.3 | 0.6 | 5.8 | 1.4 | 1.3 | 0.3 |
| 3 | .... | 97 | 62 | 9.5 | .... | 68 | 60 | 42 | 2 | 9.1 | 3.0 | .4 | .2 | 0.10 | 0 | 0 | 0 |
| 7 | .... | 3.3 | 3.3 | 1.8 | .... | .... | 73 | 50 | 4 | 1.4 | 0 | .05 | 0 | .30 | 0 | 0 | 0 |
| 10 | 24 | 2.0 | .3 | 1.7 | 45 | 90 | 37 | 14 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 0.3 | .3 | 0 | .... | 24 | 90 | 25 | .... | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | .... | .... | [a]1.3 | .... | .... | .... | [a]21 | .... | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28 | .... | .... | [a]0.2 | .... | .... | .... | [a]3.1 | .... | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 36 | .... | .... | [a]0 | .... | .... | .... | [a]0 | .... | 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 42 | .... | .... | [a]0 | .... | .... | .... | [a]0 | .... | 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[a] Inoculated by dipping plant top in liquid.

There is a very evident retardation of the rate of decline at the lower temperatures. A second series of October 23, 1918, confirms this for a still lower range of temperature. The higher temperatures seem to accelerate the decline, but the unfortunate low initial inoculation of the soil requires a repetition of the test. At the time of handling series 1, the influence of soil dryness in prolonging persistence had not been determined,

and too little attention was given to watering the pots regularly during the latter part of the experiment. But the outside pots retained moisture much better than those inside, and any difference would have been against longer persistence in them.

## INFLUENCE OF SOIL MOISTURE ON PERSISTENCE

A test was begun September 2, 1918, using ordinary potting soil. Inoculation was with a mixture of beef bouillon and potato cylinder cultures. One set of duplicate pots was kept near the saturation point by watering thoroughly every other day. A second lot was restored at each watering to the halfway point between saturation and the original air-dry condition of the soil. The third set was left unwatered. The percentages in Table VI are based on 600 inoculated punctures.

TABLE VI.—*Percentages of infection on grapefruit leaves inoculated with graded dilutions of soil solution at various intervals after the soil had been inoculated with P. citri and had been held at three moisture contents*

| Number of days between inoculation and sampling. | Soil continuously saturated. | | | | Soil moderately watered. | | | | Soil air dry. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1/1 | 1/10 | 1/100 | 1/1,000 | 1/1 | 1/10 | 1/100 | 1/1,000 | 1/1 | 1/10 | 1/100 | 1/1,000 |
| 0 | 62 | 70 | 77 | 68 | 68 | 55 | 80 | 47 | 43 | 80 | 85 | 57 |
| 1 | 54 | 23 | 38 | 32 | 57 | 38 | 18 | 43 | 55 | 83 | 47 | 42 |
| 3 | 53 | 72 | 37 | 8.7 | 67 | 60 | 45 | 21 | 67 | 78 | 71 | 70 |
| 5 | 48 | 55 | 23 | 11 | 75 | 48 | 43 | 4.3 | 65 | 53 | 7.2 | 7 |
| 7 | 2.2 | 0.8 | 0.2 | 0 | 10 | 4.2 | 1 | 0 | 11 | 3.3 | 1.2 | 3 |
| 12 | 1.5 | .7 | .3 | 0 | 0.3 | .2 | 0 | 0 | 1.8 | 4.5 | .8 | 0 |
| 17 | 0 | .3 | | | .2 | 0 | | | 3.5 | .2 | | |

The foregoing test shows no very pronounced or definite differences in rate of decrease. The slight differences tend toward lag with decrease of moisture, the moderately watered soil showing possibly less rapid decline than the saturated, and the air-dry soil showing still greater retardation.

Further tests of moderately wet soil as compared with dry were made at different times with three lots of Florida soil and are reported in Table VII. The soil for series 1 was from a "sand-soak" spot at Estero, Fla.; for series 2, from high pine land near Leesburg, Fla.; and for series 3, from intermediate pine land at Orlando, Fla. These tests were made by Miss Clara H. Hasse, of this office, during October and November, 1918, and through her courtesy are presented here. Only the 1/1 dilutions are included in Table VII, since in each series the results from higher dilutions were in accord with these. The percentages are based on inoculation of 600 punctures.

The first series indicates distinctly a retarded decline and prolonged persistence in the dry soil. The second series, with another type, shows no decided difference between the wet and dry. In the third series the initial decline was more rapid in the dry than in the wet soil.

TABLE VII.—*Percentages of infection on grapefruit leaves inoculated with 1/1 solutions of three Florida soils at various intervals after the soils had been inoculated with P. citri and had been held at two moisture contents*

| Number of days between inoculation and sampling. | Series 1. | | Series 2. | | Series 3. | |
|---|---|---|---|---|---|---|
| | Wet. | Dry. | Wet. | Dry. | Wet. | Dry. |
| 0 | 98 | 98 | 100 | 97 | 95 | 100 |
| 3 | 98 | 100 | 100 | 95 | 86 | 19 |
| 6 | 83 | 74 | 77 | 90 | 8.8 | 0.5 |
| 9 | 82 | 94 | 90 | 64 | 5.7 | .7 |
| 14 or 15 [a] | 9.7 | 93 | 63 | 23 | 0 | .3 |
| 21 | 6.3 | 40 | 1.8 | 1.7 | ..... | ..... |
| 41 or 43 | 0 | 18 | 0 | 0 | ..... | ..... |
| 50 to 54 | 0 | 0 | 0 | 0 | 0 | 0 |

[a] Where two numbers appear for days they indicate slight differences in the sampling periods for the several series.

In Table VIII two series, one set up June 12 and one July 8, 1919, are compared. Both were with soil from Orlando, Fla., of the same type but collected at different times. The inoculum for each 6-inch pot in the two series was from four potato cylinder cultures. The first series was kept well watered. The second was dried overnight after the original inoculation and kept air-dry thereafter. The percentages for the first series are averaged for four similar pots and are based on 4,000 inoculated punctures; those for the second are for three pots and are based on 3,000 inoculated punctures. These series were set up and conducted for approximately the first two months by Miss Clara H. Hasse.

TABLE VIII.—*Percentages of infection on grapefruit leaves inoculated with graded dilutions of soil solution at various intervals after the soil had been inoculated with P. citri and had been held at two moisture contents*

| Number of days between inoculation and sampling. | Series 1, moist soil. | | | | Series 2, dry soil. | | | |
|---|---|---|---|---|---|---|---|---|
| | 1/1 | 1/10 | 1/100 | 1/1,000 | 1/1 | 1/10 | 1/100 | 1/1,000 |
| 0 | 59 | 73 | 70 | 60 | 94 | 97 | 98 | 73 |
| 2 | 66 | 93 | 87 | 92 | 22 | 7.5 | 2.4 | 0.4 |
| 4 | 40 | 17 | 50 | 13 | 11 | 2.0 | 2.9 | .1 |
| 7, 6 [a] | 44 | 44 | 11 | 3.9 | 2.6 | 2.1 | .4 | 0 |
| 9, 8 | 17 | 14 | 2.5 | 1.6 | 3.9 | 2.1 | .5 | 0 |
| 11, 10 | 13 | 8.3 | 3.0 | 1.0 | 23 | 4.6 | .8 | 0 |
| 13 | 2.1 | 2.4 | .6 | .02 | 30 | 4.0 | .9 | .3 |
| 18 | 2.8 | 1.2 | .3 | .2 | 16 | 2.7 | .4 | 12 |
| 21, 23 | .6 | .4 | .03 | 0 | 32 | 9.6 | .9 | .3 |
| 33, 34 | 0 | .03 | 0 | ..... | 20 | 1.7 | .1 | 0 |
| 40, 43 | .03 | 0 | 0 | ..... | 3.7 | 1.1 | .3 | ..... |
| 52, 54 | 0 | .03 | 0 | ..... | .6 | .2 | .03 | ..... |
| 60, 62 | 0 | 0 | 0 | ..... | 1.7 | .5 | ..... | ..... |
| 69, 71 | 0 | 0 | ..... | ..... | 1.5 | .6 | ..... | ..... |
| 78, 80 | 0 | ..... | ..... | ..... | 3.0 | .9 | ..... | ..... |
| 88, 90 | 0 | ..... | ..... | ..... | .4 | ..... | ..... | ..... |
| 97, 99 | 0 | ..... | ..... | ..... | .1 | ..... | ..... | ..... |
| 106, 108 | 0 | ..... | ..... | ..... | .1 | ..... | ..... | ..... |
| 116, 118 | 0 | ..... | ..... | ..... | .05 | ..... | ..... | ..... |
| 125, 133 | 0 | ..... | ..... | ..... | .1 | ..... | ..... | ..... |
| ..., 166 | ..... | ..... | ..... | ..... | .1 | ..... | ..... | ..... |

[a] Where two numbers appear for days the first applies to series 1 and the second to series 2.

Heavy initial inoculation, frequent samplings over a long period, and inoculation at each sampling of 40 or 30 grapefruit leaves with 100 punctures each for the respective series render the results in these series especially noteworthy. In the moist series, after the fourth day, one notes a general equality of percentages on diagonals extending downward and to the left from any of the 1/1,000 figures. For example, the 1/1,000 dilution on the fourth day, the 1/100 on the seventh, the 1/10 on the ninth, and the 1/1 on the eleventh are approximately the same, indicating a nine-tenths loss in actual numbers in the soil for each sampling interval as compared with the preceding one; and this seems to hold true until the fortieth day. It may be explained that the moist series suffered much from the dropping of leaves heavily infected from the early samplings, and the resulting figures are somewhat erratic.

In the dry series there is a decided drop following the initial drying immediately after inoculation. Then follows a slow decline followed by an inexplicable increase between the tenth and thirty-fourth days. Afterwards there is an extremely gradual decline, if any, extending to the one hundred and sixty-sixth day.

On October 27, 1919, the one hundred and twelfth day of the test, a portion of the soil was removed from each of the three dry pots and moistened with sterile distilled water. The following tabulation shows the results of inoculation tests made from these moistened lots in comparison with the original dry soil. Two thousand punctures were inoculated from each lot of soil, making 6,000 for each test of moistened or of dry soil. The figures are total infections from 6,000 punctures.

| | Date of sampling. | | | | | | |
|---|---|---|---|---|---|---|---|
| | Oct. 29. | Oct. 31. | Nov. 3. | Nov. 5. | Nov. 7. | Nov. 12. | Nov. 17. |
| Dry soil | 2 | 6 | 3 | 8 | 9 | 0 | 6 |
| Moistened soil | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

The application of sterile distilled water seemingly resulted in prompt and complete extinction of *P. citri* in this dry soil which had constantly shown the presence of at least small numbers of the organism during almost four months. A repetition of the test, begun November 14, 1919, confirms these results.

That this extinction was not due to any toxic property peculiar to the distilled water was shown by a second test begun December 13, 1919, in which spring water and deep well water were used for wetting the soil. Tests on the third and seventh days were negative for all lots of moistened soil, while the dry soil continued to show the usual trace of *P. citri*.

That rate of drying would have an influence on the residuum of *P. citri* is to be expected and probably accounts for some of the irregularities

already noted in the behavior of the dry soil series when no control was exercised over the rate of drying. In a preliminary test, comparisons were made of the infective power of soil samples similarly inoculated and air-dried with different rates of rapidity at approximately the same rather warm temperature. A sample dried in less than one day gave 1.5 per cent infection, one dried in two days gave 0.1 per cent infection, and one dried in seven days gave no infection in tests made in each case immediately after drying.

An extended test of persistence in air-dry soil was made by Miss Clara H. Hasse. On October 22, 1918, soil from Winter Park, Fla., was heavily inoculated and dried as quickly as possible, in about one hour. Tests for infectiveness were made by several methods, usually by dusting the dry soil over punctured leaves which were atomized with water, the plants being later held in moist chambers. The total punctures inoculated in each test ranged from 600 to 5,000. The following percentage results were obtained:

| | Date of sampling. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Oct. 22, 1918. | Oct. 25, 1918. | Oct. 28, 1918. | Nov. 1, 1918. | Nov. 8, 1918. | Jan. 2, 1919. | June 11, 1919. | Aug. 8, 1919. | Sept. 23, 1919. | Dec. 26, 1919. |
| Percentage of infection..... | 94.1 | 68.3 | 8.5 | 2.3 | 1.5 | 0.1 | 0.24 | 0.22 | 0.48 | 0.05 |

On December 22, 1919, a portion of this soil was moistened with tap water from a deep well and was tested on the fourth and seventh days in comparison with the part remaining dry. In these tests the moistened soil gave no infection, while the dry soil continued to show traces.

## PERSISTENCE IN SOILS MADE ARTIFICIALLY ALKALINE AND ACID

Greenhouse potting soil was used in 6-inch pots. Duplicate pots were watered each with 400 cc. of water containing 1.6 cc. sulphuric acid. Two pots were watered with 400 cc. of water containing 224 cc. clear lime water prepared by slaking 25 gm. quicklime and making up to 1,000 cc. A titration test showed this lime water to be sufficient to neutralize the quantity of acid applied to the other pots. A third pair of pots was watered with 400 cc. distilled water. After standing three days all pots were equally inoculated with *P. citri*. On each sampling date litmus paper tests of the 1/1 soil washings were made, and such small amounts of lime water or diluted acid were added as seemed necessary to maintain approximately the original distinct acidity in one set and distinct alkalinity in the other. The watering of all sets was equalized. The percentage results given in Table IX are based on infections out of 2,000 inoculated punctures.

TABLE IX.—*Percentages of infection on grapefruit leaves inoculated with graded dilutions of three soil solutions at various intervals after the soils had been treated with lime water, dilute sulphuric acid, and distilled water, respectively, and inoculated with P. citri*

| Number of days between inoculation and sampling. | 1. Alkaline soil. | | | | 2. Normal soil. | | | | 3. Acid soil. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1/1 | 1/10 | 1/100 | 1/1,000 | 1/1 | 1/10 | 1/100 | 1/1,000 | 1/1 | 1/10 | 1/100 | 1/1,000 |
| 0 | 80 | 95 | 70 | 33 | 93 | 100 | 46 | 26 | 16 | 80 | 50 | 24 |
| 2 | 69 | 84 | 67 | 49 | 83 | 70 | 46 | 23 | 7.5 | 22 | 3.8 | 1.5 |
| 4 | 56 | 46 | 38 | 8.9 | 41 | 40 | 30 | 3.0 | .6 | .5 | .5 | 0 |
| 7 | 9.1 | 5.1 | 2.9 | .6 | 3.8 | 2.1 | .6 | .2 | 0 | 0 | 0 | 0 |
| 9 | 2.7 | .4 | .2 | .1 | .5 | .2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | .1 | 0 | 0 | .05 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 0 | .3 | 0 | 0 | .05 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | .2 | .5 | 0 | .05 | 0 | 0 | 0 | .05 | 0 | 0 | 0 | 0 |
| 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | 0 | 0 | ..... | ..... | 0 | 0 | ..... | ..... | 0 | 0 | ..... | ..... |
| 37 | 0 | ..... | ..... | ..... | 0 | ..... | ..... | ..... | 0 | ..... | ..... | ..... |
| 46 | 0 | ..... | ..... | ..... | 0 | ..... | ..... | ..... | 0 | ..... | ..... | ..... |

This preliminary and very artificial series indicates a slight retardation of decline in the alkaline soil and a distinct acceleration in the acid soil. In the latter, one notes the low infection percentages for the 1/1 dilution as compared with the 1/10 of the same series, or with the 1/1 of the other two series. While there is quite generally a tendency for the 1/1 dilution to give unexpectedly low results, the present instance suggests that the rather high acidity of the first wash water vehicle may play a part here in preventing infection. This matter calls for further experimentation. Since the tendency of most citrus soils is toward acidity, the evidence presented in Table IX is reassuring as to the decline of *P. citri* in such soils, notwithstanding the very unnatural conditions of the experiment.

## PERSISTENCE DEEP IN THE SOIL

The tests for downward penetration were made by placing partially dry soil in open pasteboard cylinders 3 inches in diameter and watering the surface with a strong *P. citri* suspension until the whole was saturated. Sections were made at proper intervals and samples taken with proper precautions from the axis of the soil column. For vertical ascent the cylinders were placed in a shallow pan containing the suspension of *P. citri*.

In an 8-inch column of Florida sandy soil sampled at 2-inch intervals on November 20, 1918, downward penetration was shown to be very uniform throughout. A 15-inch column of greenhouse potting soil was tested October 1, 1919, with similar practically uniform penetration, as shown by sampling at 3-inch intervals.

In Florida soil tested for vertical ascent, the capillary rise was 6 inches during four hours. Two-inch samplings showed *P. citri* to be uniformly distributed.

While testing experimental methods, it was found that the organism is readily carried in the capillary current along an absorbent cotton wick at least 10 inches.

The indication that *P. citri* may readily penetrate deep into the soil raises the question of whether conditions deep in the soil may influence the persistence of the organism differently from those near the surface. A test was made by burying 4-inch pots of inoculated potting soil in large containers, so that the pots were completely surrounded by 8 inches of soil. Samplings were made at approximately 5-day intervals. No decided difference was noted between the buried pots and similarly inoculated ones held on the greenhouse bench.

## PERSISTENCE IN AUTOCLAVED SOIL

Greenhouse potting soil in 4-inch pots was autoclaved July 15, 1918, for one hour with steam at 12 pounds pressure. When the soil was cold four autoclaved pots were inoculated, as well as four others containing similar soil not autoclaved. The percentage results in Table X are based on infection out of 1,200 inoculated punctures.

TABLE X.—*Percentages of infection on grapefruit leaves inoculated with graded dilutions of solutions from unautoclaved and autoclaved soils at various intervals after the soils had been inoculated with P. citri*

| Number of days between inoculation and sampling. | Unautoclaved soil. | | | | Autoclaved soil. | | | |
|---|---|---|---|---|---|---|---|---|
| | 1/1 | 1/10 | 1/100 | 1/1,000 | 1/1 | 1/10 | 1/100 | 1/1,000 |
| 0 | 73 | 73 | 16 | 0.9 | 44 | 3.4 | 6.2 | 0.3 |
| 4 | 60 | 35 | 7.8 | 3.7 | 60 | 68 | 13 | 15 |
| 9 | 0.9 | 0.7 | .1 | 0 | 28 | 17 | 75 | 40 |
| 14 | 0 | 0 | 0 | 0 | 9.5 | 4.3 | .8 | .7 |
| 18 | .1 | .2 | 0 | 0 | 9.2 | 13 | 9.4 | 1.3 |
| 24 | 0 | .1 | 0 | 0 | 1.9 | 1.9 | 2.1 | .2 |
| 29 | 0 | 0 | 0 | 0 | .8 | .3 | .3 | .2 |
| 35 | 0 | 0 | 0 | 0 | .4 | .1 | 0 | .1 |
| 44 | 0 | 0 | ..... | ..... | 0 | 0 | ..... | ..... |

The pots were kept on the greenhouse bench, each covered with paper. No special precautions were adopted to insure continued sterility in the autoclaved pots, if indeed the original steaming was sufficient for complete sterilization. Platings on agar at the end of the test showed miscellaneous bacteria in these pots in apparently as great numbers as in the unautoclaved ones. The autoclaved soil shows a decided lag in the decline of *P. citri*. A second series run two months later confirms this result.

## PERSISTENCE IN WATER

Water was held in cotton-stoppered flasks in 200-cc. quantities. Water from a local spring was used in comparison with distilled water. Unfortunately the flasks of autoclaved distilled water became contaminated,

as was shown by Petri dish platings soon after the series was begun, and the results from them are not included in the tabulation. The percentages in Table XI are based on infection in 1,500 punctures.

TABLE XI.—*Percentages of infection on grapefruit leaves inoculated with graded dilutions of distilled water, autoclaved spring water, and unautoclaved spring water at various intervals after the water had been inoculated with P. citri*

| Number of days between inoculation and sampling. | Distilled water. | | | | Spring water, autoclaved. | | | | Spring water, not autoclaved. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1/1 | 1/10 | 1/100 | 1/1,000 | 1/1 | 1/10 | 1/100 | 1/1,000 | 1/1 | 1/10 | 1/100 | 1/1,000 |
| 0 | 100 | 97 | 43 | 14 | 97 | 87 | 51 | 8.2 | 100 | 96 | 58 | 13 |
| 2 | 0.1 | 0 | 0 | 0 | 72 | 8.2 | 5.8 | .3 | 1.7 | 0.5 | 0.5 | 0 |
| 4 | 0 | 0 | 0 | 0 | 85 | 28 | 5.1 | .8 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | ..... | 90 | 69 | 12 | ..... | 0 | 0 | 0 | ..... |
| 11 | 0 | 0 | ..... | ..... | 58 | 25 | ..... | ..... | 0 | 0 | ..... | ..... |
| 16 | 0 | ..... | ..... | ..... | 34 | ..... | ..... | ..... | 0 | ..... | ..... | ..... |
| 21 | 0 | ..... | ..... | ..... | 44 | ..... | ..... | ..... | 0 | ..... | ..... | ..... |
| 30 | 0 | ..... | ..... | ..... | 40 | ..... | ..... | ..... | 0 | ..... | ..... | ..... |

Two other series confirmed the very rapid decline noted above in either distilled or ordinary surface water when it is nonsterile. Contrasted with this is the long persistence of moderately reduced numbers of the organism in the sterilized spring water.

The question is sharply raised, does autoclaving promote the persistence of *P. citri* in soil or water by destroying something that is deleterious or by producing something that is favorable? Autoclaving, in general, has its greatest effect in destroying the organic fauna and flora of the medium, and a subsidiary one in modifying the nutritive materials contained in it. The supposition that starvation may be the cause of the normal decline and that autoclaving the soil supplies enough available nutriment for a greatly prolonged persistence does not seem reasonable because of the disproportion between the changes that could possibly be brought about by autoclaving and the effects observed on *P. citri* persistence. Furthermore, this supposition of starvation is not adequate to explain the extinction of *P. citri* in air-dry soil when moistened.

## INHIBITORS

The deleterious effects of organisms on the development of *P. citri* is frequently observed in poured plates when fungus or bacterial contaminators entirely inhibit the development of *P. citri* for considerable distances from their limits of growth. The behavior of some of these inhibitors has been made the subject of special preliminary study.

A series of plates was prepared September 13, 1919, from beef agar rather heavily and uniformly inoculated with *P. citri*. When hard, they were inoculated in addition with a bacterium, designated inhibitor A, previously obtained from a chance contamination on a poured plate. On some plates two streaks of the inhibitor were made at right angles

across the plates; in others it was planted at the center and at four spots near the circumference; in still others it was planted abundantly over the plate. Seven days later *P. citri* was seen growing in triangular areas between the limbs of the crosses, in isolated patches with concave borders between the spots, and not at all on the plates with numerous colonies of the inhibitor. It appeared only where the distance was at least 15 to 18 mm. from the nearest border of an inhibiting colony. A hand lens and the low power of the microscope brought within range of vision two successive graded zones each about 3 mm. wide of smaller *P. citri* colonies edging the clearly visible areas. The average distance from the edge of the inhibiting colony to the *P. citri* colonies of microscopic size was about 10 mm. Repeated attempts to cultivate *P. citri* from bits of agar from this clear 10-mm. zone failed, although the abundance of the original inoculation would have made it easy to recover the organism at any point, if it were still alive. It was recovered in culture from the microscopic and the clearly visible zones, and no extension of the killing effect could be determined after a further lapse of seven days, during which time there was no apparent growth of the inhibiting colonies.

The testing of some 40 miscellaneous soil bacteria and fungi on beef agar plates by the streak or the spot method showed about one-fourth of the number to have some degree of inhibiting effect, while three seemed to stimulate or accelerate the development of *P. citri* colonies, at least at the beginning of their development.

On other media the effects of certain of these inhibitors differed from those exhibited on beef agar, the inhibiting effect being reduced or entirely lost on certain media.

Tests in the soil itself must be conducted before definite statements can be made as to the part such potential inhibitors or destroyers actually play in the decline of *P. citri* under soil conditions. However, the hypothesis that the deleterious effects on *P. citri* are brought about by certain organisms in the soil is in harmony with the experimental evidence thus far obtained and seems to be a reasonable explanation of the phenomenon.

It seems reasonable to suppose that *P. citri* can persist in dry soil partly at least because of suspended activity of deleterious organisms, and that the addition of water makes possible a renewal of their unfavorable activity.

## INFECTION OF GRAPEFRUIT ROOTS BY P. CITRI

The question naturally arises as to whether roots of citrus species are highly susceptible to citrus-canker infection. The following tests bear on this point.

On May 20, 1918, eight pots of soil were inoculated heavily with *P. citri* culture and planted with grapefruit seed, about 50 per pot. Eight other pots were similarly prepared without inoculation. In another set both seed and soil were inoculated, and in still another only the seeds were

inoculated. After two months the seedlings in all had made good growth, and there was no evidence of citrus-canker lesions on any part of the plants. In the light of present knowledge it would not have been expected that a single soil inoculation at the time of planting would persist long enough to become very effective.

On July 10, 1918, a series of pots was planted with grapefruit seed and given frequent waterings with *P. citri* suspension, on July 10, 13, 17, 20, 24, 27, and August 2. By this time the seedlings had emerged above ground, and before each subsequent watering several cuts were made through the soil with a knife to produce root wounds. Further applications of *P. citri* suspension were made August 5, 8, 10, 14, and 16. On August 31 the seedlings were removed, washed, and examined with a hand magnifier. No canker lesions were apparent on any part of the 40 plants thus examined. A test performed on grapefruit leaves with soil from these pots which had received 12 applications of heavy inoculum at close intervals gave negative results.

Direct inoculation of the roots of potted grapefruit seedlings was made as follows: On July 27, 1918, potted plants were selected with vigorous roots of about $^3/_{16}$ inch diameter extending ½ to 3 inches through the drainage holes of the pots. These roots were punctured at 10 points each, wrapped in cotton wet with *P. citri* suspension, and later placed in flats of moist, clean sand. Two weeks later infection was 40 per cent. Microscopic sections showed typical canker lesions involving the cortex. Pure cultures of *P. citri* were readily obtained by plating, and grapefruit leaves were infected therefrom. Four months later no extension of infection was apparent on the roots, most of them having continued their growth to all appearance normally. In several, however, the roots were broken at old lesions apparently following secondary decay. The plants as a whole had not suffered.

The indications are that young grapefruit roots are not readily infected except through direct wound inoculation and that the plants do not suffer from a moderate number of lesions so produced.

## SUMMARY

(1) The method of using graded dilutions of soil washings for inoculating punctured grapefruit leaves proved satisfactory for indicating the relative abundance of *P. citri* in the soil at times of sampling.

(2) Tests on many types of soil, including representative ones from citrus regions, show a very rapid decline of *P. citri* in all.

(3) This decline was retarded slightly by rendering the soil alkaline with lime water or by lowering its temperature, and more decidedly by withholding water or by previous sterilizing with steam.

(4) An extremely long persistence, in very small numbers, is noted in soil held in air-dry condition; but the organism seemingly suffers prompt extinction when water is again added.

(5) The decline is accelerated decidedly by the addition of dilute sulphuric acid or by a moderate rise in temperature.

(6) *P. citri* may easily penetrate the soil to depths ordinarily cultivated, but the normal decline seems to occur at such depths.

(7) In water the decline is more rapid than in soil. Previous sterilizing of the water has a decided effect in prolonging persistence.

(8) Certain bacteria found commonly in soils have a marked deleterious effect on *P. citri* in artificial culture media both by inhibiting growth and by killing.

(9) The presence of such deleterious organisms in soils would probably be concerned in producing a decline of *P. citri*.

(10) Young roots of grapefruit seedlings seem not to be readily infected by *P. citri* except through wounds.

## CONCLUSION

The main question at issue is whether or not *P. citri* can persist in the soil to a sufficient degree or for a long enough time to be a source of danger in the dissemination or holding over of the citrus-canker disease. The experimental evidence shows clearly that the organism undergoes a rapid and continuous decline in numbers under soil conditions that would obtain in agricultural practice. As a rule, this decline reaches the vanishing point for *P. citri* in about two weeks by the test methods employed, and it is only reasonable to suppose that the downward trend continues rapidly in such cases to absolute extinction. The potential ability of certain soil organisms to destroy *P. citri*, as shown in certain artificial culture media, lends weight to this latter supposition. Even where long-time persistence has been induced experimentally, the conditions necessary to bring it about are too extreme to make a duplication probable under natural conditions. Furthermore, the experimental methods employed for testing the infectiveness of the soil are many times more severe than would obtain under most favorable natural conditions for the spread of infection from soil to plants. All these considerations suggest that agricultural soils probably can not long retain a dangerous possibility of disseminating the citrus-canker organism.

# VARIATION OF INDIVIDUAL PIGS IN ECONOMY OF GAIN[1]

By R. C. ASHBY and A. W. MALCOMSON, *Division of Animal Husbandry, Minnesota Agricultural Experiment Station*[2]

When the initial tests with self-feeders were undertaken in 1914 the question at once arose, "What variations will appear in rations as selected by individual pigs?" To answer it 10 pigs were self-fed individually during the summer of 1915. A study of their rations has been published.[3] But in tabulating the data another factor presented itself—namely, material variations in economy of gain by the different individuals. Two similar tests have been continued in order to gain further information on this point. It is our purpose to report here the results thus far obtained.

While marked variations have been found with all groups tested, no attempt is made to explain them, because facilities have been entirely inadequate to permit a fundamental study. In one instance the junior author has made thorough type and conformation studies of 15 individuals. His data will appear in thesis form.

To date 67 individuals, representing 14 litters, have been fed individually. The experiments have been conducted during three summers and are reported as tests A, B, and C. As explained later this report includes the data on 63 pigs.

## TEST A, FEEDING PERIOD 128 DAYS

As mentioned, the records of the pigs fed in 1915 are already available. A summary for nine pigs is presented here, No. 11 being omitted because of its low final weight. For the nine pigs the average initial weight was 47.42 pounds and the average final weight 267.33 pounds. A comparison of the pigs is given in Table I.

Classified according to the degree of variation from the mean or normal grain requirement for the group:

1 pig shows a variation from the mean of more than 10 per cent.
2 pigs show a variation from the mean of between 5 and 10 per cent.
6 pigs show a variation from the mean of less than 5 per cent.

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[1] Published with the approval of the Director as Paper No. 102, Journal Series, Minnesota Agricultural Experiment Station.

[2] The authors express to Prof. H. K. Hayes their appreciation for assistance in arranging and verifying the correlation table, and to Dr. C. W. Gay and Miss Alice McFeely for helpful suggestions.

[3] ASHBY, R. C. SELF-BALANCED RATIONS BY INDIVIDUAL PIGS. *In* Amer. Soc. Anim. Prod. Proc. 1915/16 p. 197-209, illus. 1917.

TABLE I.—*Grain required to produce 100 pounds gain in pigs of test A*

[Average initial weight, 47.42 pounds; average final weight, 267.33 pounds]

| Litter. | Pig No. | Daily gain. | Grain for 100 pounds gain. | Variation from mean grain for 100 pounds gain. | |
|---|---|---|---|---|---|
| | | *Pounds.* | *Pounds.* | *Pounds.* | *Per cent.* |
| 1 | 4 | 1.908 | 402.04 | + 7.24 | 0.18 |
| 1 | 2 | 1.890 | 380.21 | −14.59 | 3.695 |
| 1 | 4 | 2.063 | 376.71 | −18.09 | 4.582 |
| 1 | 6 | 1.492 | 423.04 | +28.24 | 7.152 |
| 1 | 7 | 1.668 | 404.38 | + 9.58 | 2.425 |
| 2 | 10 | 1.603 | 430.76 | +35.96 | 9.108 |
| 2 | 12 | 1.369 | 397.99 | + 3.19 | .808 |
| 2 | 13 | 1.635 | 395.60 | + .80 | .202 |
| 2 | 14 | 1.835 | 355.31 | −39.49 | 10.002 |
| Mean grain for 100 pounds gain | | | 394.80 | | |

TEST B, FEEDING PERIODS 84 AND 100 DAYS

In 1916, 26 pigs were fed individually. Six of these which were fed on pasture plots make up group 1. Of the remaining 20, 7 which averaged 193.71 pounds each at the close of the test constitute group 2. The remaining 13 were younger pigs, except DJ 37 and P 72, which started on feed at lighter weights and averaged only 137 pounds at the close. These 13 made up group 3. The data of test B are given in Tables II to IV.

TABLE II.—*Grain required to produce 100 pounds gain in pasture-fed pigs of test B, group 1*

[Average initial weight, 34.2 pounds; average final weight, 136.9 pounds]

| Litter. | Pig No. | Daily gain. | Grain for 100 pounds gain. | Variation from mean grain for 100 pounds gain. | |
|---|---|---|---|---|---|
| | | *Pounds.* | *Pounds.* | *Pounds.* | *Per cent.* |
| DJ | 33 | 1.357 | 371.70 | +28.59 | 8.332 |
| DJ | 35 | 1.514 | 346.20 | + 3.09 | .900 |
| PY | 3 | 1.315 | 289.20 | −53.91 | 15.712 |
| DJ | 46 | 1.294 | 365.80 | +22.69 | 6.613 |
| PD | 2 | 1.250 | 325.00 | −18.11 | 5.278 |
| PD | 6 | 1.238 | 364.10 | +20.99 | 6.117 |
| Mean grain for 100 pounds gain | | | 343.11 | | |

TABLE III.—*Grain required to produce 100 pounds gain in dry-lot pigs of test B, group 2*

[Average initial weight, 42.07 pounds; average final weight, 193.71 pounds]

| Litter. | Pig No. | Daily gain. | Grain for 100 pounds gain. | Variation from mean grain for 100 pounds gain. | |
|---|---|---|---|---|---|
| | | *Pounds.* | *Pounds.* | *Pounds.* | *Per cent.* |
| DJ | 30 | 1.417 | 455.20 | +46.85 | 11.473 |
| DJ | 31 | 1.080 | 456.30 | +47.95 | 11.742 |
| DJ | 32 | 1.900 | 425.00 | +16.65 | 4.077 |
| DJ | 34 | 1.287 | 408.80 | + .45 | .110 |
| DJ | 36 | 1.940 | 382.30 | −26.05 | 6.379 |
| PY | 5 | 1.577 | 367.20 | −41.15 | 10.077 |
| PY | 6 | 1.724 | 383.40 | −24.95 | 6.109 |
| Mean grain for 100 pounds gain | | | 408.35 | | |

TABLE IV.—*Grain required to produce 100 pounds gain in dry-lot pigs of test B, group 3*

[Average initial weight, 29.6 pounds; average final weight, 137 pounds]

| Litter. | Pig No. | Daily gain. | Grain for 100 pounds gain. | Variations from mean grain for 100 pounds gain. | |
|---|---|---|---|---|---|
| | | *Pounds.* | *Pounds.* | *Pounds.* | *Per cent.* |
| DJ | 38 | 1.380 | 361.70 | −18.09 | 4.763 |
| DJ | 39 | 1.326 | 379.20 | − .59 | .155 |
| DJ | 41 | 1.258 | 399.60 | +19.81 | 5.216 |
| DJ | 42 | 1.198 | 392.20 | +12.41 | 3.267 |
| DJ | 43 | 1.397 | 406.10 | +26.31 | 6.927 |
| DJ | 44 | 1.040 | 383.10 | + 3.31 | .871 |
| PD | 1 | 1.452 | 363.30 | −16.49 | 4.341 |
| PD | 3 | 1.282 | 392.30 | +12.51 | 3.293 |
| PD | 4 | 1.052 | 378.20 | − 1.59 | .418 |
| PD | 5 | 1.175 | 431.40 | +51.61 | 13.589 |
| DJ | 37 | 1.157 | 358.70 | −21.09 | 5.553 |
| PY | 2 | 1.177 | 340.30 | −39.40 | 10.307 |
| Mean grain for 100 pounds grain | | | 379.79 | | |

If the three groups are combined, the pigs may be classified as follows on the basis of degree of variation from their respective means:

6 pigs show a variation from the mean of more than 10 per cent.
9 pigs show a variation from the mean of between 5 and 10 per cent.
10 pigs show a variation from the mean of less than 5 per cent.

## TEST C

In 1917 three tests were conducted. As before, 6 pigs were fed on pasture and 9 were carried on individual self-feeders in dry lot. In addition 16 pure-bred pigs intended for breeding animals were selected for individual feeding. Of the 15 market pigs 2 were very small at the beginning of the test and much lighter than the others at the close. For that reason they are omitted. The data for the three groups are given in Tables V to VII.

TABLE V.—*Grain required to produce 100 pounds gain in pasture-fed pigs of test C, group 1, fed 118 days*

[Average initial weight, 35.8 pounds; average final weight, 172.84 pounds]

| Litter. | Pig No. | Daily gain. | Grain for 100 pounds gain. | Variations from mean grain for 100 pounds gain. | |
|---|---|---|---|---|---|
| | | *Pounds.* | *Pounds.* | *Pounds.* | *Per cent.* |
| PB | 6 | 1.030 | 419.98 | +49.81 | 13.457 |
| PB | 7 | 1.118 | 364.84 | − 5.31 | 1.433 |
| PB | 16 | 1.239 | 365.26 | − 4.90 | 1.324 |
| PY | 1 | 1.220 | 386.25 | +16.08 | 4.345 |
| PY | 4 | 1.197 | 320.94 | −49.21 | 14.296 |
| Mean grain for 100 pounds gain | | | 370.16 | | |

TABLE VI.—*Grain required to produce 100 pounds gain in dry-lot pigs of test C, group 2, fed 118 days*

[Average initial weight, 43.62 pounds; average final weight, 185.46 pounds]

| Litter. | Pig No. | Daily gain. | Grain for 100 pounds gain. | Variations from mean grain for 100 pounds gain. | |
|---|---|---|---|---|---|
| | | *Pounds.* | *Pounds.* | *Pounds.* | *Per cent.* |
| PB | 1 | 1.42 | 420.91 | +36.82 | 9.586 |
| PB | 3 | 1.06 | 473.57 | +89.48 | 23.296 |
| PB | 9 | 1.26 | 363.43 | −20.66 | 5.378 |
| PB | 15 | 1.15 | 343.85 | −40.24 | 10.476 |
| DJ | 12 | 1.47 | 370.85 | −13.24 | 3.447 |
| DJ | 13 | 1.05 | 419.12 | +35.03 | 9.120 |
| PY | 2 | 1.11 | 340.13 | −43.96 | 11.445 |
| PY | 3 | 1.05 | 342.31 | −41.78 | 10.877 |
| Mean grain for 100 pounds gain | | | 384.09 | | |

The degrees of variation from the group means classify thus:

6 pigs show a variation from the mean of more than 10 per cent.
3 pigs show a variation from the mean of between 5 and 10 per cent.
4 pigs show a variation from the mean of less than 5 per cent.

TABLE VII.—*Grain required to produce 100 pounds gain in pasture-fed pigs of test C, group 3, fed 79 days*

[Average initial weight, 63.6 pounds; average final weight, 147.3 pounds]

| Litter. | Pig No. | Daily gain. | Grain for 100 pounds gain. | Variations from mean grain for 100 pounds gain. | |
|---|---|---|---|---|---|
| | | *Pounds.* | *Pounds.* | *Pounds.* | *Per cent.* |
| PC | 2 | 0.94 | 309.78 | −55.18 | 15.119 |
| PC | 3 | 1.01 | 394.50 | +29.54 | 8.093 |
| PC | 6 | .88 | 321.42 | −43.54 | 11.931 |
| PC | 8 | 1.06 | 315.89 | −49.07 | 13.445 |
| DJ | 2 | 1.15 | 332.96 | −32.00 | 8.768 |
| DJ | 3 | 1.18 | 396.15 | +31.19 | 8.546 |
| DJ | 5 | 1.06 | 394.40 | +29.44 | 8.066 |
| DJ | 6 | .81 | 327.81 | −37.15 | 10.170 |
| DJ | 7 | 1.21 | 367.91 | + 2.95 | .808 |
| DJ | 8 | 1.45 | 335.39 | −29.57 | 8.102 |
| DJ | 10 | 1.35 | 461.57 | +96.61 | 26.471 |
| DJ | 11 | 1.14 | 308.81 | −56.15 | 15.385 |
| PC | 13 | .84 | 442.83 | +77.87 | 21.336 |
| PC | 14 | .83 | 451.73 | +86.77 | 23.775 |
| PC | 15 | 1.09 | 309.93 | −55.03 | 15.079 |
| PC | 16 | .89 | 362.25 | − 2.71 | .742 |
| Mean grain for 100 pounds gain | | | 364.96 | | |

Note that both extremes are found in the same litter, DJ 10 and DJ 11. Wide variations appear here, but because of the comparatively short feeding period of 79 days and the low final average weight these results can not be accepted on a par with those from the preceding groups. Tabulating the results for group 3 on the basis of extent of variation from the mean, we have:

3 pigs showing a variation from the mean of more than 20 per cent.
3 pigs showing a variation from the mean of between 15 and 20 per cent.
3 pigs showing a variation from the mean of between 10 and 15 per cent.
5 pigs showing a variation from the mean of between 5 and 10 per cent.
2 pigs showing a variation from the mean of less than 5 per cent.

The occurrence and scope of variation are further emphasized by Table VIII in which the extremes from 11 litters are compared.

TABLE VIII.—*Extremes of daily gain and weight of grain required to produce 100 pounds gain in 11 litters.*

| Litter. | Pig No. | Daily gain. | Grain for 100 pounds gain. |
|---|---|---|---|
| | | *Pounds.* | *Pounds.* |
| 1 | 4 | 2.06 | 376.71 |
| 1 | 6 | 1.49 | 423.04 |
| 2 | 10 | 1.60 | 430.76 |
| 2 | 14 | 1.83 | 355.31 |
| PD | 2 | 1.25 | 325.00 |
| PD | 6 | 1.23 | 364.10 |
| DJ | 30 | 1.41 | 455.20 |
| DJ | 36 | 1.94 | 382.30 |
| DJ | 38 | 1.38 | 361.70 |
| DJ | 43 | 1.39 | 406.10 |
| PY | 1 | 1.22 | 386.25 |
| PY | 4 | 1.19 | 320.94 |
| DJ | 12 | 1.47 | 370.85 |
| DJ | 13 | 1.05 | 419.12 |
| PC | 2 | .94 | 309.78 |
| PC | 3 | 1.01 | 394.50 |
| PC | 14 | .83 | 451.73 |
| PC | 15 | 1.09 | 309.93 |
| DJ | 3 | 1.18 | 396.15 |
| DJ | 6 | .81 | 327.81 |
| DJ | 10 | 1.35 | 461.51 |
| DJ | 11 | 1.14 | 308.81 |

Of the 65 pigs an unexpectedly large number show marked variation from the normal or mean grain requirement per unit of gain.

Summing up all groups, we find:

22 pigs showing a variation from the mean of more than 10 per cent.
19 pigs showing a variation from the mean of between 5 and 10 per cent.
22 pigs showing a variation from the mean of less than 5 per cent.

On a percentage basis:

34.92 per cent exceeded 10 per cent variation.
30.15 per cent showed between 5 and 10 per cent variation.
34.92 per cent showed less than 5 per cent variation.

As stated before, no attempt is now made to explain these differing requirements, but the question of a possible correlation between the rate of gain and economy of gain naturally suggests itself. In fact, a casual inspection of the groups leads one to expect such a correlation.

In Tables IX to XV the individuals are ranked in order of efficiency both as to daily rate of gain and economy of gain.

TABLE IX.—*Rank of pigs of test A in rate and economy of gain*

| Rate of gain. | | Economy of gain. | | Rate of gain. | | Economy of gain. | |
|---|---|---|---|---|---|---|---|
| Rank. | Pig No. | Rank. | Pig No. | Rank. | Pig No. | Rank. | Pig No. |
| 1..... | 4 | 1..... | 14 | 6..... | 13 | 6..... | 1 |
| 2..... | 1 | 2..... | 4 | 7..... | 10 | 7..... | 7 |
| 3..... | 2 | 3..... | 2 | 8..... | 6 | 8..... | 6 |
| 4..... | 14 | 4..... | 13 | 9..... | 12 | 9..... | 10 |
| 5..... | 7 | 5..... | 12 | | | | |

TABLE X.—*Rank of pigs of test B, group 1, in rate and economy of gain*

| Rate of gain. | | Economy of gain. | | Rate of gain. | | Economy of gain. | |
|---|---|---|---|---|---|---|---|
| Rank. | Pig No. | Rank. | Pig No. | Rank. | Pig No. | Rank. | Pig No. |
| 1..... | DJ 35 | 1..... | PY 3 | 4..... | DJ 46 | 4..... | PD 6 |
| 2..... | DJ 33 | 2..... | PD 2 | 5..... | PD 2 | 5..... | DJ 46 |
| 3..... | PY 3 | 3..... | DJ 35 | 6..... | PD 6 | 6..... | DJ 33 |

TABLE XI.—*Rank of pigs of test B, group 2, in rate and economy of gain*

| Rate of gain. | | Economy of gain. | | Rate of gain. | | Economy of gain. | |
|---|---|---|---|---|---|---|---|
| Rank. | Pig No. | Rank. | Pig No. | Rank. | Pig No. | Rank. | Pig No. |
| 1..... | DJ 36 | 1..... | PY 5 | 5..... | DJ 30 | 5..... | DJ 32 |
| 2..... | DJ 32 | 2..... | DJ 36 | 6..... | DJ 34 | 6..... | DJ 30 |
| 3..... | PY 6 | 3..... | PY 6 | 7..... | DJ 31 | 7..... | DJ 31 |
| 4..... | PY 5 | 4..... | DJ 34 | | | | |

TABLE XII.—*Rank of pigs of test B, group 3, in rate and economy of gain*

| Rate of gain. | | Economy of gain. | | Rate of gain. | | Economy of gain. | |
|---|---|---|---|---|---|---|---|
| Rank. | Pig No. | Rank. | Pig No. | Rank. | Pig No. | Rank. | Pig No. |
| 1..... | PD 1 | 1..... | PY 2 | 7..... | DJ 42 | 7..... | DJ 44 |
| 2..... | DJ 43 | 2..... | DJ 37 | 8..... | PY 2 | 8..... | DJ 42 |
| 3..... | DJ 38 | 3..... | DJ 38 | 9..... | PD 5 | 9..... | PD 3 |
| 4..... | DJ 39 | 4..... | PD 1 | 10..... | DJ 37 | 10..... | DJ 41 |
| 5..... | PD 3 | 5..... | PD 4 | 11..... | PD 4 | 11..... | DJ 43 |
| 6..... | DJ 41 | 6..... | DJ 39 | 12..... | DJ 44 | 12..... | PY 5 |

TABLE XIII.—*Rank of pigs of test C, group 1, in rate and economy of gain*

| Rate of gain. | | Economy of gain. | | Rate of gain. | | Economy of gain. | |
|---|---|---|---|---|---|---|---|
| Rank. | Pig No. | Rank. | Pig No. | Rank. | Pig No. | Rank. | Pig No. |
| 1..... | PB 16 | 1..... | PY 4 | 4..... | PB 7 | 4..... | PY 1 |
| 2..... | PY 1 | 2..... | PB 7 | 5..... | PB 6 | 5..... | PB 6 |
| 3..... | PY 4 | 3..... | PB 16 | | | | |

TABLE XIV.—*Rank of pigs of test C, group 2, in rate and economy of gain*

| Rate of gain. | | Economy of gain. | | Rate of gain. | | Economy of gain. | |
|---|---|---|---|---|---|---|---|
| Rank. | Pig No. | Rank. | Pig No. | Rank. | Pig No. | Rank. | Pig No. |
| 1..... | DJ 12 | 1..... | PY 2 | 5..... | PY 2 | 5..... | DJ 5 |
| 2..... | PB 1 | 2..... | PY 3 | 6..... | PB 3 | 6..... | DJ 13 |
| 3..... | PB 9 | 3..... | PB 15 | 7..... | PY 3 | 7..... | PB 1 |
| 4..... | PB 15 | 4..... | PB 9 | 8..... | DJ 13 | 8..... | PB 8 |

TABLE XV.—*Rank of pigs of test C, group 3, in rate and economy of gain*

| Rate of gain. | | Economy of gain. | | Rate of gain. | | Economy of gain. | |
|---|---|---|---|---|---|---|---|
| Rank. | Pig No. | Rank. | Pig No. | Rank. | Pig No. | Rank. | Pig No. |
| 1..... | DJ 8 | 1..... | DJ 11 | 9..... | DJ 5 | 9..... | PC 16 |
| 2..... | DJ 10 | 2..... | PC 2 | 10..... | PC 3 | 10..... | DJ 7 |
| 3..... | DJ 7 | 3..... | PC 15 | 11..... | PC 2 | 11..... | DJ 5 |
| 4..... | PJ 3 | 4..... | PC 8 | 12..... | PC 16 | 12..... | PP 3 |
| 5..... | DJ 2 | 5..... | PC 6 | 13..... | PC 6 | 13..... | DJ 3 |
| 6..... | DJ 11 | 6..... | DJ 6 | 14..... | PC 13 | 14..... | PC 13 |
| 7..... | PC 15 | 7..... | DJ 2 | 15..... | PC 14 | 15..... | PC 14 |
| 8..... | PC 8 | 8..... | DJ 8 | 16..... | DJ 6 | 16..... | DJ 10 |

Selecting approximately the top half of each group, on the basis of rate of gain, we have 4 top pigs from test A; 3 from test B, group 1; 3 from test B, group 2; 6 from test B, group 3; 3 from test C, group 1; 4 from test C, group 2; and 8 from test C, group 1; making a total of 31 pigs. Of this number 19 were placed in the corresponding top halves of their respective economy columns.

In other words, slightly more than 60 per cent of the fastest-growing pigs were also distinctly economical producers. This would indicate that slightly more than one-half of the fastest-growing pigs in an average group would qualify on an economy basis.

The foregoing comparison is independent of litter relationships. Selecting and comparing the fastest-growing pig with the slowest-gainer from the same litter, we find the following results from 12 litters:

In 6 cases the fastest growing pig was most economical.
In 3 cases the fastest growing pig was least economical.

In 3 cases the fastest growing pig was moderately economical.
In 2 cases the slowest-growing pig was most economical.
In 5 cases the slowest-growing pig was least economical.
In 5 cases the slowest-growing pig was moderately economical.

Apparently this indicates a certain degree of correlation between the characters under discussion. As a more accurate determination of correlation between rate of gain and economy of gain the data are correlated in Table XVI. For this purpose the variations, both in rate of gain and economy of gain, are reduced to a percentage basis.

XVI.—*Correlation between rate of gain and economy of gain*

| Economy of gain (in percentages of the mean.) | Rate of gain (in percentages of the mean). | | | | | | | | | | | | | | Total. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 70 | 75 | 80 | 85 | 90 | 95 | 100 | 105 | 110 | 115 | 120 | 125 | 130 | 135 | |
| 85 | ... | ... | ... | ... | 1 | ... | 2 | 2 | 1 | ... | ... | ... | ... | ... | 6 |
| 90 | ... | 1 | ... | 1 | 1 | 3 | 1 | 1 | 1 | ... | ... | ... | ... | 1 | 10 |
| 95 | ... | ... | ... | ... | ... | 2 | ... | 1 | 3 | 1 | 1 | 2 | ... | ... | 10 |
| 100 | ... | ... | 2 | 3 | ... | 3 | ... | 2 | 1 | 2 | ... | ... | ... | ... | 13 |
| 105 | ... | ... | ... | 1 | ... | 3 | 1 | 2 | 1 | ... | 1 | ... | ... | ... | 9 |
| 110 | 1 | ... | ... | ... | 2 | 2 | 2 | ... | 1 | ... | 1 | ... | ... | ... | 9 |
| 115 | ... | ... | ... | ... | 1 | 1 | ... | ... | ... | ... | ... | ... | ... | ... | 2 |
| 120 | ... | ... | 1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | 1 |
| 125 | ... | ... | 1 | ... | 1 | ... | ... | ... | ... | ... | ... | ... | 1 | ... | 3 |
| Total | 1 | 1 | 4 | 5 | 6 | 14 | 6 | 8 | 8 | 3 | 3 | 2 | 1 | 1 | ... |

$r = -0.452 \pm 0.068$.

The resultant coefficient of correlation ($r = -0.452 \pm 0.068$) shows a distinct negative correlation between rate of gain and economy of gain, entirely disproving the apparent relation shown by Tables IX to XV. The differing requirements per unit of gain are of much practical moment. As has been noted, the variation in rate of gain shows a standard deviation in percentage of $9.57 \pm 0.58$ and an average deviation of 8.01 per cent.

## POSSIBLE APPLICATION

Pointing out applications before establishing final conclusions is as dangerous as selling property without possession of title, but a consideration of probabilities is ever in order.

It is safe to emphasize again the danger of conclusions based on feeding trials where small groups are the experimental units. If average individual variations of 7 per cent are at all common, a statement in a former Oregon Experiment Station bulletin[1] that—

> the reader should therefore hesitate at putting too much weight on differences amounting to less than 10 per cent

carries much weight.

[1] WITHYCOMBE, James, POTTER, Ermine L., and SAMSON, George R. EXPERIMENTS IN SWINE FEEDING. Oreg. Agr. Exp. Sta. Bul. 127, p. 5. 1915.

However, our main interest lies in the possibility of utilizing this factor of variation, making it a definite factor in the breeder's support. Is it a hereditary character? How is it transmitted? Can the breeder through careful testing and selective mating develop or produce a strain that is more economical in feeding or pure for the quality of economy in production? Can he produce a line that is homozygous for this characteristic? Extreme results are not to be expected, but even a moderate saving, if constant, would be a marked achievement.

In this connection a feature of Danish agricultural practice is very interesting. An article[1] describing it came to hand as our data were being tabulated, and a brief quotation is pertinent in this connection:

> There is, however, quite another group of qualities which must be kept in mind in connection with swine-breeding, but which cannot be estimated with sufficient accuracy with the naked eye, namely, the quality of the bacon and the thrivingness and growing energy of the pigs.
>
> The Experimental Laboratory has, during a long period of years, carried out experiments with regard to the offspring of stud animals in the breeding centers which afford reliable and helpful information as to the powers of transmission of qualities possessed by the stud animals in regard to the qualities mentioned. It is the breeding centers which supply the material for these experiments.
>
> The owner of each recognized breeding center is bound to supply on an average two young pigs from selected sow annually to the Experiment Stations, and as there are about 900 selected sows (757 Danish and 147 Yorkshire), the stations have at their disposal a good deal of material. For pecuniary and other reasons they have found it necessary to confine themselves to about 1,000 test animals per annum. Nevertheless, the experiments are on a big scale such as is scarcely equalled elsewhere.
>
> The young pigs are supplied at the age of seven or eight weeks. Each experiment pen contains four full-blooded sisters and brothers. All the pens receive the same food mixture in weighed proportions, and the animals themselves are weighed at regular intervals. The experiments finish when the abattoir weight is reached . . . The result is made use of in the selection of stud animals, those being preferred whose descendants have shown the highest degree of thrivingness and growth energy and the best bacon.

This is a good plan and doubtless characteristic of the results obtained through Danish agricultural cooperative organization, though just how each pen could contain four litter mates when only two pigs are sent from each litter is a bit puzzling.

Of recent years the possibility of a "register of merit" for meat animals has received considerable attention. If more thorough investigation corroborates our results and should it be found possible to develop families or strains that are more economical producers, no sounder basis of preferment could be desired.

If selection along this line will achieve results, we believe it desirable to put the work on an individual basis from the start. The Danish plan deals with pen averages which our data show to be somewhat unreliable so far as indicating the true performance of the individuals concerned.

[1] MÖRKEBERG, Peter Aug. THE PRESENT POSITION AND FUTURE PROSPECTS OF SWINEBREEDING IN DENMARK. *In* Dept. Agr. and Tech. Instr. Ireland Jour., v. 17, no. 1, p. 46-47. 1916.

But since the "breeding centers" have been a factor for at least 20 years, and doutless the "experimental laboratory" has been in operation a good part of that time, the continuation of the plan attests its efficiency. By adopting the individual as the unit we eliminate the probable inaccuracy of pen averages and hope to have taken at least one step in devising a practical method for measuring the efficiency of meat-producing animals.

# PRODUCTION OF CONIDIA IN GIBBERELLA SAUBINETII[1]

By JAMES G. DICKSON, *Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*, and *Assistant Professor of Plant Pathology, University of Wisconsin*, and HELEN JOHANN, *Assistant Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.*

The scab fungus *Gibberella saubinetii* (Mont.) Sacc., which attacks wheat, corn, rye, barley, and oats, has been considered as having a vegetative stage and two spore stages. The conidial and perithecial development terminates the active vegetative period. Strains producing abundant perithecia have been described as developing only a few conidia in scattered, sporodochia-like masses.

Cultural studies with a large number of strains of *G. saubinetii* show that in nature, as well as in artificial culture, this species produces conidia at two different periods during its development. Wollenweber[2] suggests this when he states that—

> on steamed potato tuber the conidia form a short-lived pionnotes. The conidia of this pionnotes rapidly swell, separate into cells, germinate, and produce new conidia, which anastomose and form a stroma, while in the other species mentioned the conidia remain perfect, dry out, and are long-lived.

The first period of conidial production is in connection with the early mycelial growth of the culture, while the second occurs at the termination of the vigorous vegetative development. These later conidia are produced in definite sporodochia and are the only conidia generally described for this species. The production of perithecia is the final stage in the development of the culture.

During the summer of 1919, single-spore cultures were made by the authors from sporodochial conidia and ascospores taken from stock cultures and from wheat heads, wheat culms, and cornstalks collected in the field. These specimens were obtained from a number of widely separated points in the central and eastern States. Spores from all sources were placed in hanging drops of distilled water and sterile tap water, on poured plates of potato-dextrose agar and soil decoction agar, and on sterile soil. The subsequent development of the fungus was then studied at frequent intervals.

[1] The investigations upon which this paper is based were conducted as a cooperative project between the Office of Cereal Investigations of the Bureau of Plant Industry and the Wisconsin Agricultural Experiment Station.

[2] WOLLENWEBER, H. W. IDENTIFICATION OF SPECIES OF FUSARIUM OCCURRING ON THE SWEET POTATO, IPOMOEA BATATAS. *In* Jour. Agr. Research, v. 2, no. 4, p. 278. 1914.

Journal of Agricultural Research,
Washington, D. C.
uj

Vol. XIX, No. 5
June 1, 1920
Key No. G-194

The spores, both conidia and ascospores, behaved alike in germination. They germinated, as described by Wollenweber, by imbibing water, increasing the number of septa (fig. 1, A, C), and forming several mycelial strands from the different cells (fig. 1, C). When the cultures were grown in a saturated atmosphere, conidia were cut off from lateral branches of mycelial strands in 24 hours (fig. 1, B, D). In 48 hours a copious conidial production took place in definite sporodochia-like clusters (fig. 1, E). On extremely moist plates these clumps occasionally massed together to form a pionnotes. As mycelial development

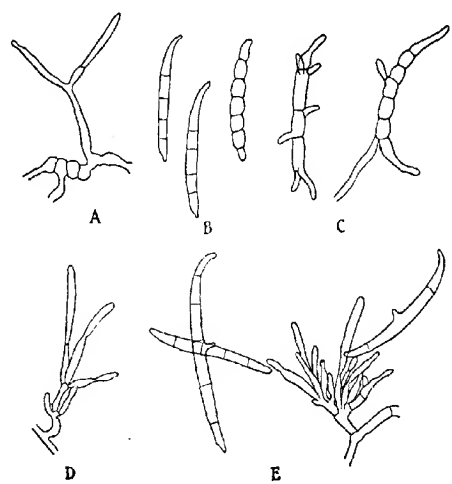


FIG. 1.—Conidial production in *Gibberella saubinetii* (Mont.) Sacc.: A, Ascospores from cornstalk, germinated in distilled water, producing conidia in three days; B, D, typical conidia and conidiophore from a 28-hour-old hanging drop culture from a conidium from A; C, germinating conidia from a 52-hour-old plate culture; E, conidiophore and germinating conidia from a 47-hour-old colony in a Van Tieghem cell. This colony was three generations from an ascospore. Potato-dextrose agar acidified with lactic acid was used unless otherwise stated.

progressed, new conidial masses developed and thus gradually increased the size of the pionnotes.

The conidia were pushed off the conidiophore before septation was completed, and new conidia formed in their place (fig. 1, E). Septation was completed after the conidia had been separated from the conidiophore. The conidia became swollen, septation increased, and germination took place in from 6 to 12 hours after leaving the conidiophore (fig. 1, B, C, E). When the cultures were moderately crowded and moisture and temperature conditions were suitable, all these conidia germinated, forming a stroma; and conidia development ceased until the final development of sporodochial conidia several weeks later. If, however, the conidia were transferred to a suitable medium and were not

overcrowded, they germinated, forming hyphae which bore masses of conidia within two days as previously described for the sporodochial conidia and ascospores. This conidial production went on indefinitely, if the culture did not dry or become crowded. The ninth generation of conidia from a single ascospore was produced in 20 days by transferring each successive generation to new plates of potato-dextrose agar. These conidia were produced only when the spores were transferred to a favorable medium and kept in a moist, warm atmosphere. When the temperature was lowered or when the culture became dry the conidia did not germinate but remained inert on the surface of the culture. Spores kept in this manner were rather resistant to both desiccation and low temperatures. Germination was obtained after several weeks' storage at temperatures of about 3° to 4° C., as well as when stored under dry conditions at room temperature.

Conidia were produced in two days from mycelium plated from infected root and stem tissues as well as from plated conidia and ascospores. Tissues infected with *G. saubinetii* were surface-sterilized and placed on potato-dextrose agar in poured plates. Conidia appeared on the developing mycelium two days after plating and were present in conspicuous sporodochia-like masses the third day. These conidia were identical with those formed on the mycelium from either ascospores or conidia.

The conidia formed during the vegetative development were 4 to 5 septate (fig. 1, B, E) and were of the same shape and size as the sporodochial conidia.

Inoculations on wheat plants showed that these conidia were as virulent in producing scab on wheat as were either sporodochial conidia or the vegetative mycelium. The spores germinated and caused infection within the same temperature range as the sporodochial conidia.

The work here reported shows that repeated crops of conidia of *G. saubinetii* can be produced in abundance in short periods of time from ascospores, sporodochial conidia, vegetative conidia, or mycelium, when favorable moisture and temperature conditions obtain. This ability of the wheatscab organism to produce virulent spores in abundance in short periods of time has an important bearing on the development of wheatscab epidemics.